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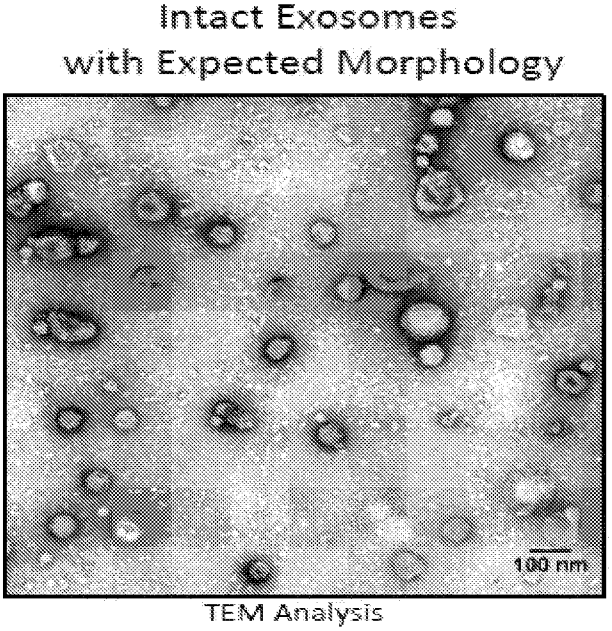

FIG. 1A

(57) Abstract: Skin care compositions comprising milk derived exosomes loaded with single skin peptide or a combination of skin peptides and methods for use in skin treatment are described.

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[Continued on next page]

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EXOSOME DELIVERY OF SKIN CARE PEPTIDES

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EXOSOME DELIVERY OF SKIN CARE PEPTIDES

Cross –Reference to Related Application

[0001] The present application claims the benefit of U.S. Provisional Patent Application Nos. 62/700,526 filed on July 19, 2018 and 62/782,438 filed December 20, 2018, which are hereby incorporated by reference in their entireties.

Field of Invention

[0002] Skin care compositions comprising milk derived exosomes loaded with single skin peptide or a combination of skin peptides and methods for use in skin treatment are described.

Incorporation By Reference

[0003] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

Background

[0004] There are two main causes of skin aging: (i) internal where levels of collagen and elastin naturally decrease as an individual ages, and (ii) external, where environmental factors such as UV exposure, pollution and tobacco use cause damage to skin. Facial skin wrinkles yield significant psychological distress worldwide in an era of aging population demographics.

[0005] One of the major causes of wrinkles, UV sun damage, triggers a loss of skin pliability and elasticity via secreted elastases and collagenases that degrade the major skin extracellular matrix (ECM) proteins, elastin and collagen. UV-induced inflammation mediators like cytokines and chemokines may also play a cooperative role, due to their association with release of free radicals and ECM-remodeling proteases.

[0006] The most common interventions available for treatment of facial wrinkles include face-lifts, laser surgery, skin peels, and injection therapies, such as BOTOX®. Such methods however, may result in complications, are often painful, and must be repeated with time.

[0007] Non-invasive remedies include topical formulations consisting of alpha/beta hydroxy, retinoic acids, argireline, and vitamins. However, none of these methods completely eliminate wrinkles, and require multiple, and often expensive treatments. Some topical formulations may act as irritants to the skin, to elicit wound healing responses, but do not successfully replenish the thinning skin with adequate proteins for treatment and/or prevention of age-related defects.

[0008] In current years, many new skin care peptides have been developed that are able to stimulate or increase the proliferation of collagen, elastin and fibronectin. However, effective transdermal skincare peptide delivery remains elusive. Consequently, there are no skin care compositions that effectively and consistently deliver skincare peptides to damaged or aged skin tissue, e.g. dermis and/or epidermis.

Summary of the Disclosure

[0009] Provided herein is an exosome comprising at least one skin care peptide. Also provided herein is a milk-derived exosome comprising at least one skin care peptide. In one embodiment, the milk is bovine milk. In some embodiments, the skin care peptide is Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-EEMQRRAD); Copper Tripeptide-1 (Copper-GHK); Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2, Tetrapeptide-21 or any combination thereof. In one embodiment, at least one of the skin care peptide is Palmitoyl Pentapeptide-4. In one embodiment, at least one of the skin care peptide is Palmitoyl Tetrapeptide-7. In one embodiment, at least one of the skin care peptide is Palmitoyl Tripeptide-38. In one embodiment, at least one of the skin care peptide is Palmitoyl Tripeptide-1. In one embodiment, at least one of the skin care peptide is Palmitoyl Hexapeptide-12. In a further embodiment, provided herein is a composition as described above and a physiologically acceptable medium.

[0010] Provided herein is an anti-wrinkle cosmetic composition comprising an effective amount of exosomes, wherein the exosomes comprise at least one skin care peptide. Also provided herein is an anti-aging cosmetic composition comprising an effective amount of exosomes,

wherein the exosomes further comprise at least one skin care peptide. In an embodiment, the compositions are topical compositions. In a further embodiment, the skin care peptide is Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-EEMQRRAD); Copper Tripeptide-1 (Copper-GHK); Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2, Tetrapeptide-21 or any combination thereof. In one embodiment, at least one of the skin care peptide is Palmitoyl Pentapeptide-4. In one embodiment, at least one of the skin care peptide is Palmitoyl Tetrapeptide-7. In one embodiment, at least one of the skin care peptide is Palmitoyl Tripeptide-38. In one embodiment, at least one of the skin care peptide is Palmitoyl Tripeptide-1. In one embodiment, at least one of the skin care peptide is Palmitoyl Hexapeptide-12. In one embodiment, the compositions further comprise an emulsifier. In one aspect, the emulsifier is Lipowax D. In another embodiment, the compositions comprise a preservative. In one aspect, the preservative is Mikrokill. In another aspect, the preservative is 1% Mikrokill.

[0011] Further provided herein is a method of obtaining milk-derived exosome loaded with skin care peptides comprising: isolating exosomes from milk; and introducing at least one skin care peptide directly into the isolated exosomes. In one embodiment, introducing at least one skin care peptide into the isolated exosomes comprises freezing and thawing the isolated exosomes and at least one skin care peptide. In another embodiment, introducing at least one skin care peptide into the isolated exosomes comprises coincubating the isolated exosomes and at least one skin care peptide. In an embodiment, introducing at least one skin care peptide into the isolated exosomes comprises sonicating the isolated exosomes and at least one skin care peptide. In a further embodiment, introducing at least one skin care peptide into the isolated exosomes comprises extrusion of the isolated exosomes and at least one skin care peptide.

[0012] In a further embodiment, the milk is bovine milk. In a further embodiment, the skin care peptide is Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-EEMQRRAD); Copper Tripeptide-1 (Copper-GHK);

Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2, Tetrapeptide-21 or any combination thereof. In one embodiment, at least one of the skin care peptide is Palmitoyl Pentapeptide-4. In one embodiment, at least one of the skin care peptide is Palmitoyl Tetrapeptide-7. In one embodiment, at least one of the skin care peptide is Palmitoyl Tripeptide-38. In one embodiment, at least one of the skin care peptide is Palmitoyl Tripeptide

[0013] Also provided herein is a method of treating skin comprising topically applying to skin of a subject in need thereof, a composition comprising an effective amount of exosomes, wherein the exosomes comprise at least one skin care peptide. In one embodiment, the composition is applied to facial skin. In another embodiment, the composition is applied to a fine line or wrinkle. In an embodiment, the composition is a cream or a lotion. In one embodiment, the composition moisturizes skin. In another embodiment, the composition increases the expression of collagen 1. In yet another embodiment, the composition increases the expression of fibronectin. In one embodiment, a subject in need thereof is a non-responder.

[0014] In a further embodiment, the composition is applied at least once a day. In another embodiment, the composition is applied before sleep. In one embodiment, the composition is non-comedogenic. In a further embodiment, the skin is erythemic, sensitive, or inflamed skin,

[0015] In a further embodiment, the skin care peptide is Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-EEMQRRAD); Copper Tripeptide-1 (Copper-GHK); Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2, Tetrapeptide-21 or any combination thereof. In one embodiment, at least one of the skin care peptide is Palmitoyl Pentapeptide-4. In one embodiment, at least one of the skin care peptide is Palmitoyl Tetrapeptide-7. In one embodiment, at least one of the skin care peptide is Palmitoyl Tripeptide-38. In one embodiment, at least one of the skin care peptide is Palmitoyl Tripeptide.

[0016] Further provided herein is a method of delivering at least one skin care peptide to a dermis layer of skin comprising applying a composition comprising an effective amount of exosomes to skin, wherein the exosomes comprise at least one skin care peptide. In one

embodiment, the composition is applied to facial skin. In another embodiment, the composition is applied to a fine line or wrinkle. In an embodiment, the composition is a cream or a lotion. In one embodiment, the composition moisturizes skin. In another embodiment, the composition increases the expression of collagen 1. In yet another embodiment, the composition increases the expression of fibronectin. In one embodiment, a subject in need thereof is a non-responder.

[0017] In a further embodiment, the composition is applied at least once a day. In another embodiment, the composition is applied before sleep. In one embodiment, the composition is non-comedogenic. In a further embodiment, the skin is erythemic, sensitive, or inflamed skin,

[0018] In a further embodiment, the skin care peptide is Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-EEMQRRAD); Copper Tripeptide-1 (Copper-GHK); Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2, Tetrapeptide-21 or any combination thereof. In one embodiment, at least one of the skin care peptide is Palmitoyl Pentapeptide-4. In one embodiment, at least one of the skin care peptide is Palmitoyl Tetrapeptide-7. In one embodiment, at least one of the skin care peptide is Palmitoyl Tripeptide-38. In one embodiment, at least one of the skin care peptide is Palmitoyl Tripeptide.

Brief Description of the Drawings

[0019] The features of the present disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0020] Figure 1A-C depicts characteristics of isolated bovine milk exosomes. Figure 1A is a Transmission Electron Microscopy (TEM) image showing intact exosomes with expected morphology. Figure 1B is a graph depicting the results of Nanosight analysis of isolated bovine

milk exosome particle size. Each line depicts bovine milk exosomes isolated from different experiments. Figure 1C is a western blot depicting markers detected on the exosomes, e.g. CD9, CD63 and CD81.

[0021] Figure 2 depicts the bioactivity of Pal-Tri-38 loaded into bovine milk exosomes. Figures 2A-C depict expression levels of collagen, elastin, and fibronectin following the treatment of fibroblast cells with Pal-Tri-38 loaded exosomes. Figures 2D-E depict expression levels of collagen and fibronectin following the treatment of keratinocytes with Pal-Tri-38 loaded exosomes.

[0022] Figure 3 depicts the bioactivity of Pal-Tri-1 loaded into bovine milk exosomes. Figures 3A-C depict expression levels of collagen, elastin, and fibronectin following the treatment of fibroblast cells with Pal-Tri-1 loaded exosomes. Figures 3D-E depict expression levels of collagen and fibronectin following the treatment of keratinocytes with Pal-Tri-1 loaded exosomes.

[0023] Figure 4 depicts the bioactivity of Pal-Tetra-7 loaded into bovine milk exosomes. Figures 4A-C depict expression levels of collagen, elastin, and fibronectin following the treatment of fibroblast cells with Pal-Tetra-7 loaded exosomes. Figures 4D-E depict expression levels of collagen and fibronectin following the treatment of keratinocytes with Pal-Tetra-7 loaded exosomes.

[0024] Figure 5 depicts the bioactivity of Pal-Penta-4 loaded into bovine milk exosomes. Figures 5A-C depict expression levels of collagen, elastin, and fibronectin following the treatment of fibroblast cells with Pal-Penta-4 loaded exosomes. Figures 5D-E depict expression levels of collagen and fibronectin following the treatment of keratinocytes with Pal-Penta-4 loaded exosomes.

[0025] Figure 6 depicts the bioactivity of Pal-Hexa-12 loaded into bovine milk exosomes. Figures 6A-C depict expression levels of collagen, elastin, and fibronectin following the treatment of fibroblast cells with Pal-Hexa-12 loaded exosomes. Figures 6D-E depict expression levels of collagen and fibronectin following the treatment of keratinocytes with Pal-Hexa-12 loaded exosomes.

[0026] Figure 7 depicts anti-aging gene expression in non-responders when treated with various exosomes loaded with skin peptides. Figure 7A is a graphical representation of collagen I expression in non-responder donor fibroblasts when treated with Pal-Tri1 loaded milk exosomes. Figure 7B is a graphical representation of collagen I expression in non-responder donor keratinocytes when treated with Pal-Tri38 loaded milk exosomes. Figure 7C is a graphical representation of collagen I expression in non-responder donor fibroblasts when treated with different concentrations of Pal-Penta4 loaded milk exosomes.

[0027] Figure 8 depicts uptake efficiencies of different concentrations of exosomes (untreated) or loaded exosomes in keratinocytes or fibroblasts. Figures 8A and B are graphical representations of Pal-Tri38 loaded exosomes uptake into keratinocyte and fibroblast at different concentrations. The Pal-Tri38 loaded exosomes were obtained using co-incubation, freeze thaw, extrusion or sonication. Figures 8C and D are graphical representations of Pal-Tetra7 loaded exosomes uptake into keratinocyte and fibroblast at different concentrations. The Pal-Tetra7 loaded exosomes were obtained using co-incubation, freeze thaw, extrusion or sonication. Controls for all experiments were Dio labeled PBS and exosome only labeled with Dio.

[0028] Figure 9A depicts penetrance of labeled skin peptides delivered by loaded milk exosomes into all three skin layers, including the dermis, of reconstituted skin from various donors. Skin peptide alone remains in the stratum corneum. Figure 9B depicts penetrance of labeled skin peptides delivered by loaded milk exosomes in all 3 skin layers, including the dermis of adult skin explants. Skin peptide alone remains in the stratum corneum.

Detailed Description

[0029] The following description and examples illustrate embodiments of the invention in detail. It is to be understood that this invention is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this invention, which are encompassed within its scope.

[0030] All terms are intended to be understood as they would be understood by a person skilled in the art. Unless defined otherwise, all technical and scientific terms used herein have the same

meaning as commonly understood by one of ordinary skill in the art to which the disclosure pertains.

[0031] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0032] Although various features of the invention may be described in the context of a single embodiment, the features may also be provided separately or in any suitable combination. Conversely, although the invention may be described herein in the context of separate embodiments for clarity, the invention may also be implemented in a single embodiment.

[0033] The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, e.g., to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present disclosure, the preferred materials and methods are described herein. Accordingly, the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0034] In this application, the use of the singular includes the plural unless specifically stated otherwise. It must be noted that, as used in the specification, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms, such as "include", "includes," and "included," is not limiting.

[0035] Reference in the specification to "some embodiments," "an embodiment," "one embodiment" or "other embodiments" means that a particular feature, structure, or characteristic described in connection with the embodiments is included in at least some embodiments, but not necessarily all embodiments, of the inventions.

[0036] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps. It is

contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

[0037] The compositions of the invention and methods for their use can "comprise," "consist essentially of," or "consist of" any of the ingredients disclosed throughout the specification. "Consisting essentially of" means that inclusion of additional ingredients in the compositions do not materially affect the multi-beneficial properties of the composition comprising exosomes loaded with skin care peptide(s).

[0038] The term "about" in relation to a reference numerical value and its grammatical equivalents as used herein can include the numerical value itself and a range of values plus or minus 10% from that numerical value. For example, the amount "about 10" includes 10 and any amounts from 9 to 11. For example, the term "about" in relation to a reference numerical value can also include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% or 0.5% from that value or any range therein.

[0039] By "isolated" is meant the removal of an exosome from its natural environment. By "purified" is meant that a given exosome, whether one that has been removed from nature or synthesized and/or amplified under laboratory conditions, has been increased in purity, wherein "purity" is a relative term, not "absolute purity." It is to be understood, however, that the exosome may be formulated with diluents or adjuvants or excipients and still for practical purposes be isolated.

[0040] As used herein "cosmetically" or "cosmetic" means a process involving a treatment intended to restore or improve the skin or the body's appearance.

[0041] The term "effective," as that term is used in the specification and/or claims, means adequate to accomplish a desired, expected, or intended result, for example, firming/toning the skin, increasing the skin's elasticity, reducing the appearance of dark spots or aged spots, evening out the skin's tone, reducing the appearance of fine lines and wrinkles, reducing other signs of premature skin aging, and reducing the appearance of expression lines. In other embodiments, the term "effective" also means adequate to accomplish a desired, expected, or intended result, for example, firming/toning the skin, increasing the skin's elasticity, reducing the appearance of

dark spots or aged spots, evening out the skin's tone, reducing the appearance of fine lines and wrinkles, reducing other signs of premature skin aging, and reducing the appearance of expression lines in non-responders. In other embodiments, the term “effective” further means consistent delivery or penetrance of skin care peptides to skin tissue, e.g. dermis and/or epidermis.

[0042] As used herein "exosomes" are defined as cell-derived vesicles that are present in many and perhaps all biological fluids, including milk, blood, urine, and cultured medium of cell cultures, possessing a diameter of between about 30 to about 100 nm, which diameter is larger than LDL, but much smaller than, for example, red blood cells. Exosomes may be released from the cell when multivesicular bodies fuse with the plasma membrane or they may be released directly from the plasma membrane. “Milk-derived exosomes” can be isolated from milk obtained from breast milk of mammals such as but not limited to: humans, bovine or equine.

[0043] The term “milk” is used herein to describe the opaque liquid that contains proteins, fats, lactose, and various vitamins and minerals and that is produced by the mammary glands of mature female mammals including, but not limited to, after the mammals have given birth to provide nourishment for their young. In some embodiments, the term "milk" can include colostrum, or the liquid that is secreted by the mammary glands of mammals at the time of parturition. Milk can be obtained from human, cow, goat, sheep, pig, monkey, dog, cat, rat, camel, mouse, hamster, guinea pig, or the like. Preferred examples include milk obtained from human or cow.

[0044] “Milk-derived exosome” is an exosome contained in milk and obtained or isolated from milk.

[0045] As used herein, “skin care peptide(s)” refer to short peptides comprising 2-50 amino acids in length that are capable of penetrating the stratus corneum, epidermis and/or dermis. In some embodiments, such peptides are modified to promote skin absorption with a fatty acid such as a palmitoyl group, phospholipids, or triglycerides. Skin care peptides can modulate fibronectin, collagen, elastin and/or melanin RNA, DNA and/or protein expression. Such skin care peptides can be effective at improving the skin firmness or skin elasticity. Skin care peptides as referred to herein, can be any skin peptide known or believed to be effective at

reducing the appearance of fine lines and wrinkles, at improving skin firmness, improving skin elasticity and /or can repair sun damage to the skin (i.e. reduce sun spots, photo aged skin). Skin care peptides that are contemplated herein may be suitable for cosmetic use (e.g. not regulated drug products active ingredients that require clinical testing). In certain embodiments, the skin care peptides loaded in exosomes such as milk exosomes for delivery to stratus corneum, epidermis and/or dermis.

[0046] The term "cosmetic composition" or simply "composition" according to the present invention, concerns a formulation which may be used for cosmetic purposes or as a base for one or more pharmaceutical ingredients. These also include cosmetics, personal care products and pharmaceutical preparations. It is also possible that these formulations may be used for two or more purposes at the same time.

[0047] The term “aging” as it refers to skin includes development of fine lines and wrinkles, loss of elasticity, increased sagging, loss of firmness, loss of color evenness (tone), coarse surface texture, and mottled pigmentation. Other measurable changes which occur as skin ages or endures chronic environmental insult include a general reduction in cellular and tissue vitality, reduction in cell replication rates, reduced cutaneous blood flow, reduced moisture content, accumulated errors in structure and function, and a reduction in the skin's ability to remodel and repair itself. Many of the above alterations in appearance and function are caused by changes in the outer epidermal layer of the skin, while others are caused by changes in the lower dermis. Regardless of the stimulus for skin damage, when damage occurs, numerous natural and complex biochemical mechanisms are set into motion in attempts to repair the damage.

[0048] The term "wrinkle" or "wrinkling" refers to both fine wrinkling and coarse wrinkling. Fine wrinkling or fine lines refers to superficial lines and wrinkles on the skin surface. Coarse wrinkling refers to deep furrows, particularly deep lines/wrinkles on the face and around the eyes, including expression lines such as frown lines and wrinkles, forehead lines and wrinkles, crow's feet lines and wrinkles, nasolabial fold and marionette lines and wrinkles. Forehead lines and wrinkles refer to superficial lines and/or deep furrows on skin of the forehead. Crow's feet lines and wrinkles refer to superficial lines and/or deep furrows on skin around the eye area. Marionette lines and wrinkles refer to superficial lines and/or deep furrows on skin around the mouth. Wrinkles can be assessed for number, length, and depth of the lines.

[0049] An “anti-wrinkle cosmetic composition” comprises a formulation which may be used to reduce appearance of wrinkles on facial skin, hand skin, or neck skin. In some embodiments, reducing the appearance of fine lines or wrinkles, improving skin tone evenness, or improving the texture of skin in a target region is determined by comparison of the skin in the target region that has fine lines, wrinkles, uneven skin tone, or rough skin texture prior to application of the composition to the skin in the target region after application of the product. An “anti-aging composition" comprises a formulation which may be used to reduce the appearance of age spots, fine lines, wrinkles, loss of elasticity, increased sagging, loss of firmness, loss of color evenness (tone), coarse surface texture, and mottled pigmentation. In some embodiments, the skin in the target region is evaluated 1, 2, 3, 4, 5, 6, or 7 days, 2, 3, 4, or 5 weeks; or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months, or any range therein, after the first application of the composition. In some embodiments, the skin in the target region is evaluated 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours, or any range therein, after the first application of the composition. In some embodiments, the composition is applied hourly, daily, weekly, or monthly. In some embodiments, the composition is applied 1, 2, 3, 4, or more times daily. Such comparisons can also be made for the other properties as described herein which can also be impacted by application of the described composition. In addition, the assays described herein can be used to evaluate a particular property at the target region.

[0050] “Topical application” means to apply or spread a composition onto the surface of keratinous tissue. “Topical skin composition" includes compositions suitable for topical application on keratinous tissue. Such compositions are typically dermatologically-acceptable in that they do not have undue toxicity, incompatibility, instability, allergic response, and the like, when applied to skin. Topical skin care compositions of the present invention can have a selected viscosity to avoid significant dripping or pooling after application to skin.

[0051] "Keratinous tissue" includes keratin-containing layers disposed as the outermost protective covering of mammals and includes, but is not limited to, skin, hair and nails.

[0052] The terms "inhibiting," "reducing," "treating," or any variation of these terms, when used in the claims and/or the specification includes any measurable decrease or complete inhibition to achieve a desired result.

[0053] "Subject" as used herein refers to a mammalian subject. Exemplary subjects can be humans, apes, dogs, pigs, cattle, cats, horses, goats, sheep, rodents and other mammalians that can benefit from the skin care or cosmetic compositions disclosed herein. Exemplary human subjects can be male and/or female. A "subject in need thereof" as referred to herein is a subject who needs to restore or improve the skin or the body's appearance. In another embodiment, a "subject in need thereof" is a non-responder with skin or body appearance that does not respond or change when treated with exosome only, with skin care peptide(s) only or with currently available skin care formulations. In one aspect, a non-responder subject who is treated with exosome only, with skin care peptide(s) only or with currently available skin care formulations, demonstrates no changes in the expression of anti-aging genes such as but not limited to collagen, elastin, or fibronectin.

[0054] Exosomes

[0055] Exosomes are membrane vesicles released from many mammalian cell types. Exosomes are also present in all biological fluids or sample including but not limited to the following bodily fluids: peripheral blood, ascites, cerebrospinal fluid (CSF), sputum, saliva, bone marrow, synovial fluid, aqueous humor, amniotic fluid, cerumen, breast milk, broncheoalveolar lavage fluid, semen (including prostatic fluid), Cowper's fluid or pre-ejaculatory fluid, female ejaculate, sweat, fecal matter, hair, tears, cyst fluid, pleural and peritoneal fluid, pericardial fluid, lymph, chyme, chyle, bile, interstitial fluid, menses, pus, sebum, vomit, vaginal secretions, mucosal secretion, stool water, pancreatic juice, lavage fluids from sinus cavities, bronchopulmonary aspirates or other lavage fluids. A biological sample may also include the blastocyl cavity, umbilical cord blood, or maternal circulation that may be of fetal or maternal origin.

[0056] Exosomes are typically 30-100 nm in diameter and may contain a variety of proteins, DNAs, mRNAs, and miRNAs. In some embodiments, the exosomes described herein do not contain any substantial amount of nucleic acids. The methods to produce empty exosomes (empty of genetic material) are known by one skilled in the art; including UV-exposure, mutation of proteins that carry RNA into exosomes, as well as electroporation and chemical treatments to open pores in the exosomal membranes. The methods can also include mutation/deletion of any protein that can modify loading of any nucleic acid into exosomes. Typically, exosomes display the same membrane orientation as the cell of origin.

[0057] Exosomes may be concentrated or isolated from a biological sample using size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfiuidic separation, commercially available protein purification kits, or combinations thereof. Size exclusion chromatography, such as gel permeation columns, centrifugation or density gradient centrifugation, and filtration methods can be used. For example, exosomes can be isolated by differential centrifugation, anion exchange and/or gel permeation chromatography, sucrose density gradients, organelle electrophoresis, magnetic activated cell sorting (MACS), or with a nanomembrane ultrafiltration concentrator. Various combinations of isolation or concentration methods can be used.

[0058] Depending on the biological sample, highly abundant proteins, such as milk fat globules (MFG), casein, albumin and immunoglobulin, may hinder isolation of exosomes. For example, exosomes may be isolated from a biological sample using a system that utilizes multiple antibodies that are specific to the most abundant proteins found in blood. Such a system can remove up to several proteins at once, thus unveiling the lower abundance species such as cell-of-origin specific exosomes. This type of system can be used for isolation of exosomes from biological samples such as milk, blood, cerebrospinal fluid, urine and/or saliva.

[0059] The isolation of exosomes from a biological sample may also be enhanced by high abundant protein removal methods as described in Chromy et al. J. Proteome Res 2004; 3: 1120-1127. In another embodiment, the isolation of exosomes from a biological sample may be enhanced by removing serum proteins using glycopeptide capture as described in Zhang et al, Mol Cell Proteomics 2005; 4: 144-155. In addition, exosomes from a biological sample such as urine may be isolated by differential centrifugation followed by contact with antibodies directed to cytoplasmic or anti-cytoplasmic epitopes as described in Pisitkun et al., Proc Natl Acad Sci USA, 2004; 101: 13368-13373.

Isolation of Milk derived exosomes

[0060] In one preferred embodiment, the exosomes of the presently-disclosed subject matter are isolated in a manner that allows for the isolation of exosomes in amounts greater than about: 50, 100, 150, 200, 250, 300, 350, 400, 450 or 500 mg of exosomal protein per 100 ml of milk. In

one embodiment, the isolation of exosomes can be achieved by centrifuging raw (i.e., unpasteurized milk or colostrum) at high speeds to isolate the exosomes. In this regard, in some embodiments, a method of isolating exosomes is further provided that includes the steps of: obtaining an amount of milk (e.g., raw milk or colostrum); and subjecting the milk to a series of sequential centrifugations configured to yield greater than about 50 mg of exosomal protein per 100 ml of milk. In some embodiments, the sequential centrifugations yield greater than 300 mg of exosomal protein per 100 ml of milk. In some embodiments, the series of sequential centrifugations comprises a first centrifugation at 20,000xg at 4°C for 30 min, a second centrifugation at 100,000xg at 4°C for 60 min, and a third centrifugation at 120,000xg at 4°C for 90 min. In another embodiment, a pre-clearance step to remove milk fat globules (MFG) is performed. In some embodiments, the isolated exosomes can then be stored at a concentration of about 5 mg/ml to about 10 mg/ml or any appropriate concentration to prevent coagulation and allow the isolated exosomes to effectively be used for the encapsulation of one or more skin care peptides. In some embodiments, the isolated exosomes are passed through a 0.22μm filter to remove any coagulated particles as well as microorganisms, such as bacteria.

[0061] In one embodiment, the lipid fraction, cell fraction, and casein fraction are removed from milk to prepare a whey (milk serum) fraction. An exosome fraction is collected from the obtained whey fraction. Preparation of exosomes from whey is described in published references, for example, Izumi H. et al., J. Dairy Sci., 98:2920-2933, 2015. For example, acetic acid can be added to milk to precipitate casein. The exosome fraction can be collected by using a known method such as ultracentrifugation, filtration, density gradient centrifugation, sucrose cushion, ultrafiltration, continuous flow electrophoresis, and chromatography. In one aspect, the whey fraction is centrifuged at 30,000 to 1,000,000xg, for example, at 100,000xg, for 60 to 120 minutes. The supernatant is removed and an exosome fraction is obtained.

[0062] An enriched population of exosomes can be obtained. For example, exosomes may be concentrated or isolated from a biological sample using size exclusion chromatography, density gradient centrifugation, differential centrifugation, nanomembrane ultrafiltration, immunoabsorbent capture, affinity purification, microfluidic separation, or combinations thereof.

[0063] Size exclusion chromatography, such as gel permeation columns, centrifugation or density gradient centrifugation, and filtration methods can be used. For example, exosomes can

be isolated by differential centrifugation, anion exchange and/or gel permeation chromatography (for example, as described in U.S. Pat. Nos. 6,899,863 and 6,812,023), sucrose density gradients, organelle electrophoresis (for example, as described in U.S. Pat. No. 7,198,923), magnetic activated cell sorting (MACS), or with a nanomembrane ultrafiltration concentrator. The quality of isolated exosomes can be determined via transmission electron microscopy (TEM), atomic force microscopy (AFM) or scanning electron microscopy (SEM). Expected characteristics include rounded vesicles with expected size in the range of 50-150 nm. The buoyant density of milk exosomes can be determined using density gradient centrifugation. Appropriate values should be for examples 1.0 g/ml, 1.05g/ml, 1.11g/ml, 1.12g/ml, 1.13g/ml, 1.14g/ml, 1.15g/ml, 1.16g/ml, 1.17g/ml, 1.18g/ml, 1.19g/ml, 1.20g/ml. In addition, assays (e.g. Western Blot) can be conducted to detect exosomal markers such as but not limited to, HC Class I protein, LAMP1, CD9, CD63 or CD81.

[0064] Once the exosomes are isolated, they can be stored at about: -80°C, -20°C or 4°C for 1 week, 2 weeks, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months or 24 months. The isolated exosomes can also be stored for 1 year, 2 years, 3 years, 4 years or 5 years prior to use. The isolated exosomes can be stored at about: -80°C, -70°C, -60°C, -50°C, -40°C, -30°C, -20°C, -10°C, -5°C, 0°C, 1°C, 2°C, 3°C, 4°C, 5°C, 6°C, 7°C, 8°C, 9°C, 10°C, 11°C, 12°C, 13°C, 14°C, 15°C, 16°C, 17°C, 18°C, 19°C, 20°C, 21°C, 22°C, 23°C, 24°C, 25°C, 26°C, 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, 34°C, 35°C, 36°C, 37°C, 38°C, 39°C or 40°C.

Skincare peptide(s)

[0065] Skincare peptides can be matricin peptides, carrier peptides, peptide mimetics or neurotransmitter-inhibiting peptides, enzyme inhibitor peptides, defensing peptides, or structural protein digestion peptides (e.g. keratin peptide). The peptide or peptides may be optically pure or may be formed from the L or D isomers or a mixture of these isomers. In the case of a peptide or peptidic compound formed from coded amino acids, the L isomers which are those present in the natural state may be preferred. "At least one skin care peptide" or "a skin care peptide" or grammatical variations thereof is used interchangeably herein refers to a specific skin care peptide. It is understood that when referring to at least one skin care peptide encapsulated by or

loaded onto an exosome, the exosome can comprise one or multiple skin care peptide(s) of the specific skin care peptide. In other embodiments, where combinations of skin care peptides (i.e. "one or more skin care peptides") are contemplated, the exosome can comprise one or a combination of specific skin care peptides.

[0066] Examples of matricins peptides include Carnosine, Copper tripeptide, Trifluoroacetyl-tripeptide-2, Tripeptide-10 citrulline, Acetyl tetrapeptide-5, Acetyl tetrapeptide-9, Acetyl tetrapeptide-11, Tetrapeptide PKEK, Tetrapeptide-21, Hexapeptide, Hexapeptide-11, Palmitoyl pentapeptide-4, Palmitoyl tripeptide-3/5, Palmitoyl tetrapeptide-7, Palmitoyl hexapeptide-12, Palmitoyl oligopeptide, Palmitoyl tripeptide-1, Pentamide-6.

[0067] Examples of carrier peptides include Copper tripeptide, Manganese tripeptide-1.

[0068] Examples of peptide mimetics include Acetyl hexapeptide-3, Pentapeptide-18, Pentapeptide-3, Tripeptide-3.

[0069] Examples of enzyme inhibitor peptides include Soybean peptide, Silk fibroin peptide, Black rice oligopeptides.

[0070] Examples of defensins include LL-37, a 37-residue antimicrobial peptide starting with a pair of leucines.

[0071] Exemplary peptides that are suitable for cosmetic use can be utilized, alone or in various combinations, include but are not limited to: Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38 (Palmitoyl Tripeptide-38 is a dioxygenated lipopeptide, also referred to as Palmitoyl-Lysyl-Dioxymethionyl-Lysine; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Palmitoyl Tetrapeptide-7 (Palmitoyl-GQPR); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-EEMQRRAD); Copper Tripeptide-1 (Copper-GHK); Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2; Tetrapeptide-21.

Methods of Loading Exosomes with Skincare Peptide(s)

[0072] "A loaded exosome" or a "loaded milk-derived exosome" or grammatical variations thereof is used interchangeably herein to refer to exosomes whose lipid bilayer surrounds one or more skin care peptide(s). The concentration of skin care peptides used in the below described methods of loading can be at least: 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.25, 0.50, 0.625, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.50, 2.75, 3.0, 3.25, 3.50, 3.75, 4.0, 4.25, 4.5, 4.75 or 5.0 mg/mL in combination with exosome concentration of at least: 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14} or 10^{15} or any range in between. In another embodiment, the concentration of skin care peptides used in the below described methods of loading can be at least: 0.005, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.25, 0.50, 0.625, 0.75, 1.0, 1.25, 1.50, 1.75, 2.0, 2.25, 2.50, 2.75, 3.0, 3.25, 3.50, 3.75, 4.0, 4.25, 4.5, 4.75 or 5.0 mg/mL in combination with exosome concentration of at least: 1 $X10^{13}$, 1.5 $X10^{13}$, 2 $X10^{13}$, 2.5 $X10^{13}$, 3 $X10^{13}$, 3.5 $X10^{13}$, 4 $X10^{13}$, 4.5 $X10^{13}$, 5 $X10^{13}$, 5.5 $X10^{13}$, 6 $X10^{13}$, 6.5 $X10^{13}$, 7 $X10^{13}$, 7.5 $X10^{13}$, 8 $X10^{13}$, 8.5 $X10^{13}$, 9 $X10^{13}$, 9.5 $X10^{13}$, or 10^{14}. Following any of the loading methods described below, the loaded exosome can be purified from any free, unloaded skin care peptides using any methods known to a person of skill in the art. In certain aspects, milk-derived exosomes, in particular non-human based milk-derived exosomes can be advantageous. In one aspect, bovine milk-derived exosomes is preferred.

[0073] In some embodiments, exosomes can be loaded prior to isolation from a biological fluid. For example, at least one or more skin care peptide(s) can be incubated with the biological fluid at 37°C for an appropriate time before isolation of the exosome. A variety of buffers commonly used for biological samples may be used for incubation of the exosomes including phosphate, acetate, citrate and TRIS buffers. The pH of the buffer may be any pH that is compatible with the exosomes, but a typical range is from 6 to 8. The buffer may have a pH from 4 to 10, 4 to 6, 4 to 8, 6 to 10, 6 to 8, or 8 to 10. The salt concentration can be any concentration that is compatible with the sample, but typically ranges from 10 mM to 500 mM. The salt concentration may be 10 mM to 500 mM, 10 mM to 50 mM, 10 mM to 100 mM, 10 mM to 200 mM, 10 mM to 300 mM, 10 mM to 400 mM, 50 mM to 500 mM, 100 mM to 500 mM, 200 mM to 500 mM, 300 mM to 500 mM, or 400 mM to 500 mM.

[0074] In other embodiments, the isolated exosome or milk-derived exosome can be loaded with one or more skin care peptide(s) by methods that include but are not limited to the following:

electroporation, sonication, incubation at room temperature, saponin-treatment, freeze-thaw cycles or extrusion.

[0075] In some embodiments, the encapsulation of one or more skin care peptides within the exosomes can be achieved by mixing the one or more of the skin care peptides with isolated exosomes in a suitable solvent, such as ethanol. After a period of incubation sufficient to allow the skin care peptide to become encapsulated during the incubation period, the loaded exosome mixture can then be subjected to a low-speed centrifugation (e.g., 10,000xg) to remove any unbound or non-loaded skin care peptide and one or more high-speed centrifugation centrifugations to isolate the loaded exosome(s).

[0076] In other embodiments, isolated exosomes can be incubated with one or more skin care peptides at room temperature or at 37°C for at least: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 18, 20, 21, 22, 23, 24 hours. In alternative embodiments, isolated exosomes can be incubated with one or more skin care peptides at room temperature or at 37°C for at least: 1, 2, 3, 4, 5, 6, 7 days. In certain aspects, the isolated exosomes can be diluted in PBS or any saline solution prior to incubation. The loaded exosomes can then be purified.

[0077] In another embodiment, isolated exosomes can be mixed with one or more skin care peptides and treated with DMSO or saponin at concentrations of at least: 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0. 7.5. 8.0, 8.5, 9.0, 9.5, or 10 %. In other embodiments, isolated exosomes can be incubated with one or more skin care peptides in DMSO or saponin at room temperature or at 37°C for at least:10, 20, 30, 40, 50, 60, 70, 80, 90, 100 hours. In certain embodiments, isolated exosomes can be incubated with one or more skin care peptides in DMSO or saponin at room temperature or at 37°C for at least: 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 hours. The loaded exosomes can then be purified.

[0078] In one embodiment, isolated exosomes can be incubated with one or more skin care peptides at room temperature or at 37°C for at least: 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 mins and then rapidly frozen at -80° C. This mixture can be then be thawed at RT. The freeze-thaw cycle can be repeated at least: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 times. Following each freeze-thaw cycle or at the end of multiple freeze-thaw cycles, the loaded exosome can be

allowed healing time of up to: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours. The loaded exosomes can then be purified.

[0079] In one embodiment, isolated exosomes can be mixed with one or more skin care peptides and sonicated. Sonication conditions can differ by % power, number of cycles, number of pulses and timing of pulses. For example, the Qsonica Sonicator Q700 (Fisher Scientific, Hampton, NH, USA) or any appropriate sonication machine may be used. In some aspects, the mixture can be sonicated at: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100% power. In other aspects the mixture can be sonicated for 10X, 20X, 30X, 40X, 50X, 60X, 70X, 80X, 90X, 100X pulses every at least: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 seconds. In between each pulse, the mixture can be allowed to cool down or heal for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30 minutes. In certain aspects, after the sonication procedure is complete, the loaded exosome can be allowed healing time of up to: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours. The loaded exosomes can then be purified.

[0080] In one embodiment, isolated exosomes can be mixed with one or more skin care peptides and extruded through an extruder such as Avanti Lipids extruder (Avanti Polar lipids Inc., Alabaster, AL, USA) with 200 nm-pores diameter or polycarbonate (PC) membrane filters with pore size of at least 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60, 0.65, 0.70, 0.75, 0.80, 0.85, 0.90, 0.95 or 1 μm. The extrusion can be performed at least: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 times. The loaded exosomes can then be purified.

Assays

[0081] The efficacy of any one of the compositions disclosed throughout the specification and claims can be determined by methods known to those of ordinary skill in the art. The following are non-limiting assays, in addition to those described in the Examples, can be used in the context of the present invention. It should be recognized that other testing procedures can be used, including, for example, objective and subjective procedures.

[0082] Gene Expression

[0083] Gene expression assays to measure the expression of anti-aging genes such as but not limited to collagen, elastin, or fibronectin can be used to measure response to the compositions described herein. Gene expression can be induced by incubation of the compositions described herein with fibroblast or keratinocyte donor cells. Effect on gene expression can be quantified by comparing composition treated cells with skin care peptide only or with media only treatment. The compositions as described herein will be considered to be effective if there is about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100 fold difference in levels of gene expression compared to appropriate controls. Alternatively, the compositions as described herein will be considered to be effective if there is about: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100 fold difference in levels of gene expression in non-responder donor cells. Non-responder donor cells are characterized as donor cells that do not demonstrate an effect on gene expression when treated with exosome only, with skin care peptide(s) only or with currently available skin care formulations.

[0084] Erythema Assay:

[0085] An assay to measure the reduction of skin redness can be evaluated using a Minolta Chromometer. Skin erythema may be induced by applying a 0.2% solution of sodium dodecyl sulfate on the forearm of a subject. The area is protected by an occlusive patch for 24 hrs. After 24 hrs, the patch is removed and the irritation-induced redness can be assessed using the a* values of the Minolta Chroma Meter. The a* value measures changes in skin color in the red region. Immediately after reading, the area is treated with a composition of the present invention. Repeat measurements are taken at regular intervals to determine the formula's ability to reduce redness and irritation.

[0086] Skin Moisture/Hydration Assay:

[0087] Skin moisture/hydration benefits can be measured by using impedance measurements with the Nova Dermal Phase Meter. The impedance meter measures changes in skin moisture content. The outer layer of the skin has distinct electrical properties. When skin is dry it conducts electricity very poorly. As it becomes more hydrated increasing conductivity results. Consequently, changes in skin impedance (related to conductivity) can be used to assess changes

in skin hydration. The unit can be calibrated according to instrument instructions for each testing day. A notation of temperature and relative humidity can also be made. Subjects can be evaluated as follows: prior to measurement they can equilibrate in a room with defined humidity (e.g., 30-50%) and temperature (e.g., 68-72°C.). Three separate impedance readings can be taken on each side of the face, recorded, and averaged. The T5 setting can be used on the impedance meter which averages the impedance values of every five seconds application to the face. Changes can be reported with statistical variance and significance.

[0088] Skin Clarity and Reduction in Freckles and Age Spots Assay:

[0089] Skin clarity and the reduction in freckles and age spots can be evaluated using a Minolta Chromometer. Changes in skin color can be assessed to determine irritation potential due to product treatment using the a* values of the Minolta Chroma Meter. The a* value measures changes in skin color in the red region. This is used to determine whether a composition is inducing irritation. The measurements can be made on each side of the face and averaged, as left and right facial values. Skin clarity can also be measured using the Minolta Meter. The measurement is a combination of the a*, b, and L values of the Minolta Meter and is related to skin brightness, and correlates well with skin smoothness and hydration. Skin reading is taken as above. In one non-limiting aspect, skin clarity can be described as L/C where C is chroma and is defined as $(a^2+b^2)^{1/2}$.

[0090] Skin Dryness, Surface Fine Lines, Skin Smoothness, and Skin Tone Assay:

[0091] Skin dryness, surface fine lines, skin smoothness, and skin tone can be evaluated with clinical grading techniques. For example, clinical grading of skin dryness can be determined by a five point standard Kligman Scale: (0) skin is soft and moist; (1) skin appears normal with no visible dryness; (2) skin feels slightly dry to the touch with no visible flaking; (3) skin feels dry, tough, and has a whitish appearance with some scaling; and (4) skin feels very dry, rough, and has a whitish appearance with scaling. Evaluations can be made independently by two clinicians and averaged.

[0092] Clinical Grading of Skin Tone Assay:

[0093] Clinical grading of skin tone can be performed via a ten point analog numerical scale: (10) even skin of uniform, pinkish brown color. No dark, erythremic, or scaly patches upon examination with a hand held magnifying lens. Microtexture of the skin very uniform upon touch; (7) even skin tone observed without magnification. No scaly areas, but slight discolorations either due to pigmentation or erythema. No discolorations more than 1 cm in diameter; (4) both skin discoloration and uneven texture easily noticeable. Slight scaliness. Skin rough to the touch in some areas; and (1) uneven skin coloration and texture. Numerous areas of scaliness and discoloration, either hypopigmented, erythremic or dark spots. Large areas of uneven color more than 1 cm in diameter. Evaluations were made independently by two clinicians and averaged.

[0094] Clinical Grading of Skin Smoothness Assay:

[0095] Clinical grading of skin smoothness can be analyzed via a ten point analog numerical scale: (10) smooth, skin is moist and glistening, no resistance upon dragging finger across surface; (7) somewhat smooth, slight resistance; (4) rough, visibly altered, friction upon rubbing; and (1) rough, flaky, uneven surface. Evaluations were made independently by two clinicians and averaged.

[0096] Skin Smoothness and Wrinkle Reduction Assay With Methods Disclosed in Packman et al. (1978):

[0097] Skin smoothness and wrinkle reduction can also be assessed visually by using the methods disclosed in Packman et al. (1978). For example, at each subject visit, the depth, shallowness and the total number of superficial facial lines (SFLs) of each subject can be carefully scored and recorded. A numerical score was obtained by multiplying a number factor times a depth/width/length factor. Scores are obtained for the eye area and mouth area (left and right sides) and added together as the total wrinkle score.

[0098] Skin Firmness Assay with a Hargens Ballistometer:

[0099] Skin firmness can be measured using a Hargens ballistometer, a device that evaluates the elasticity and firmness of the skin by dropping a small body onto the skin and recording its first two rebound peaks. The ballistometry is a small lightweight probe with a relatively blunt tip (4

square mm-contact area) was used. The probe penetrates slightly into the skin and results in measurements that are dependent upon the properties of the outer layers of the skin, including the stratum corneum and outer epidermis and some of the dermal layers.

[0100] Skin Softness/Suppleness Assay with a Gas Bearing Electrodynamometer:

[0101] Skin softness/suppleness can be evaluated using the Gas Bearing Electrodynamometer, an instrument that measures the stress/strain properties of the skin. The viscoelastic properties of skin correlate with skin moisturization. Measurements can be obtained on the predetermined site on the cheek area by attaching the probe to the skin surface with double-stick tape. A force of approximately 3.5 gm can be applied parallel to the skin surface and the skin displacement is accurately measured. Skin suppleness can then be calculated and is expressed as DSR (Dynamic Spring Rate in gm/mm).

[0102] Appearance of Lines and Wrinkles Assay with Replicas:

[0103] The appearance of lines and wrinkles on the skin can be evaluated using replicas, which is the impression of the skin's surface. Silicone rubber like material can be used. The replica can be analyzed by image analysis. Changes in the visibility of lines and wrinkles can be objectively quantified via the taking of silicon replicas form the subjects' face and analyzing the replicas image using a computer image analysis system. Replicas can be taken from the eye area and the neck area, and photographed with a digital camera using a low angle incidence lighting. The digital images can be analyzed with an image processing program and the area of the replicas covered by wrinkles or fine lines was determined.

[0104] Surface Contour of the Skin Assay with a Profilometer/Stylus Method:

[0105] The surface contour of the skin can be measured by using the profilometer/stylus method. This includes either shining a light or dragging a stylus across the replica surface. The vertical displacement of the stylus can be fed into a computer via a distance transducer, and after scanning a fixed length of replica a cross-sectional analysis of skin profile can be generated as a two-dimensional curve. This scan can be repeated any number of times along a fixed axis to generate a simulated 3-D picture of the skin. Ten random sections of the replicas using the stylus technique can be obtained and combined to generate average values. The values of interest

include Ra which is the arithmetic mean of all roughness (height) values computed by integrating the profile height relative to the mean profile height. Rt which is the maximum vertical distance between the highest peak and lowest trough, and Rz which is the mean peak amplitude minus the mean peak height. Values are given as a calibrated value in mm. Equipment should be standardized prior to each use by scanning metal standards of know values. Ra Value can be computed by the following equation: R_a=Standardize roughness; l_m=the traverse (scan) length; and y=the absolute value of the location of the profile relative to the mean profile height (x-axis).

[0106] MELANODERM™ Assay:

[0107] In other non-limiting aspects, the efficacy of the compositions of the present invention can be evaluated by using a skin analog, such as, for example, MELANODERM™ Melanocytes, one of the cells in the skin analog, stain positively when exposed to L-dihydroxyphenyl alanine (L-DOPA), a precursor of melanin. The skin analog, MELANODERM™ can be treated with a variety of bases containing the compositions and whitening agents of the present invention or with the base alone as a control. Alternatively, an untreated sample of the skin analog can be used as a control.

[0108] ORAC Assay:

[0109] Oxygen Radical Absorption (or Absorbance) Capacity (ORAC) of the aromatic skin-active ingredients and compositions can also be assayed by measuring the antioxidant activity of such ingredients or compositions. This assay can quantify the degree and length of time it takes to inhibit the action of an oxidizing agent such as oxygen radicals that are known to cause damage cells (e.g., skin cells). The ORAC value of the aromatic skin-active ingredients and compositions can be determined by methods known to those of ordinary skill in the art (see U.S. Publication Nos. 2004/0109905 and 2005/0163880; Cao et al. (1993)), all of which are incorporated by reference). In summary, the assay described in Cao et al. (1993) measures the ability of antioxidant compounds in test materials to inhibit the decline of B-phycoerythrin (B-PE) fluorescence that is induced by a peroxyl radical generator, AAPH.

[0110] Matrix Metalloproteinase Enzyme Activity (MMP3; MMP9) Assay:

[0111] An in vitro matrix metalloprotease (MMP) inhibition assay. MMPs are extracellular proteases that play a role in many normal and disease states by virtue of their broad substrate specificity. MMP3 substrates include collagens, fibronectins, and laminin; while MMP9 substrates include collagen VII, fibronectins and laminin. Using Colorimetric Drug Discovery kits from BioMol International for MMP3 (AK-400) and MMP-9 (AK-410), this assay is designed to measure protease activity of MMPs using a thiopeptide as a chromogenic substrate (Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC2H5)5,6. The MMP cleavage site peptide bond is replaced by a thioester bond in the thiopeptide. Hydrolysis of this bond by an MMP produces a sulfhydryl group, which reacts with DTNB [5,5'-dithiobis(2-nitrobenzoic acid), Ellman's reagent] to form 2-nitro-5-thiobenzoic acid, which can be detected by its absorbance at 412 nm (c=13,600 M-lcm-1 at pH 6.0 and above 7).

[0112] Anti-Aging Assays

[0113] To measure anti-aging effects of the compositions described herein, the following assays can be used: stratum corneum transit time, as measured by the rate of loss of skin color following treatment with dihydroxy acetone, (2) barrier integrity, as measured by temporary trans-epidermal water loss (TEWL) following overnight treatment with occlusive patches containing the test material, and (3) tests for actual facial skin benefit, including measurements of skin moisture on the face, long term TEWL, skin firmness, number of wrinkles around the canthus (the corner of the eye), texture (roughness) of the canthus skin, skin scales, and expert grading evaluations.

Cosmetic Composition

[0114] In one embodiment, a composition or a cosmetic composition comprising exosomes that are loaded with a skincare peptide or peptides, as the active ingredient, combined with an appropriate excipient, i.e. a physiologically or dermatologically acceptable medium, is described. The composition of the present invention can also be used in many cosmetic products including, but not limited to, sunscreen products, sunless skin tanning products, hair products, finger nail products, moisturizing creams, skin benefit creams and lotions, softeners, day lotions, gels, ointments, foundations, night creams, lipsticks, cleansers, toners, masks, or other known cosmetic products or applications. Additionally, the cosmetic products can be formulated as

leave-on or rinse-off products. In certain aspects, the compositions of the present invention are stand-alone products.

[0115] The term "dermatological or physiological medium" means, but is not restricted to, an aqueous or aqueous-alcoholic solution, a water in oil emulsion, an oil in water emulsion, a micro-emulsion, an aqueous gel, an anhydrous gel, a serum, a dispersion of vesicles. "Dermatologically acceptable" or "physiologically acceptable" means that the compositions or compounds derived are suitable for use in contact with mammal, and more particularly human, mucosal membranes, nails, scalp, head hair, body hair and skin without risk of toxicity, incompatibility, instability, allergic response or others.

[0116] In one aspect, the dermatologically acceptable vehicle comprises at least 50% by weight of water, 3 to 10% by weight of glycerin, 3 to 10% by weight of butylene glycol, 1 to 3% by weight of glyceryl stearate, 1 to 5% by weight of caprylic/capric triglyceride, and 1 to 5% by weight of hydrogenated polydecene. In another aspect, the dermatologically acceptable vehicle comprises water, glycerin, butylene glycol, glyceryl stearate, caprylic/capric triglyceride, and hydrogenated polydecene. The ratio of water to glycerin can be important for skin types. In some instances, the water:glycerine ratio is about 5:1 to about 20:1 (or 6:1, 7:1, 8:1, 9:1, 10:1, 11:1, 12:1, 13:1, 14:1, 15:1, 16:1, 17:1, 18:1, 19:1 or any range therein) based on the total weight of the composition.

[0117] According to other beneficial features, the cosmetic or dermopharmaceutical composition according to the invention may incorporate one or more additional active ingredients. The additional active ingredients may be selected from brightening, anti-redness agents, sunscreens and UV organic or inorganic filters, hydration, moisturizing, humectants, exfoliants, anti-wrinkle, anti-ageing, slimming, anti-acne, anti-inflammatory, anti-oxidant, radical scavenger, self-tanning, depilation or shave, hair growth moderator, tightening agents, peptides and vitamins. The active ingredients can be obtained from plant material.

[0118] More specifically, the exosomes loaded with a skin care peptide or peptides may be combined with at least one compound selected from vitamin B3 compounds like niacinamide or tocopherol, retinol, hexamidine, alpha-lipoic acid, resveratrol and DHEA (dehydroepiandrosterone).

[0119] In addition, the composition may also include betaine, glycerol, Actimoist Bio 2™ (Active organics), AquaCacteen™ (Mibelle AG Cosmetics), Aquaphyline™ (Silab), AquaregulK™ (Solabia), Carciline™ (Greentech), Codiavelane™ (Biotech Marine), Dermaflux™ (Arch Chemicals, Inc), Hydra'Flow™ (Sochibo), Hydromoist L™ (Symrise), RenovHyal™ (Soliance), Seamoss™ (Biotech Marine), Essenskin™ (Sederma), Moist 24™ (Sederma), Argireline™, the commercial name of acetyl hexapeptide-3 (Lipotec), spilanthol or an extract of Acmella oleracea known by the name Gatuline Expression™ (EP 1722864), extract of Boswellia serrata known by the name Boswellin™, Deepaline PVB™ (Seppic), Syn-AKE™ (Pentapharm), Ameliox™, Bioxilift™ (Silab) and mixtures thereof.

[0120] Among other plant extracts which can be combined with the compounds of the invention, there may more particularly be mentioned extracts of Ivy, in particular English Ivy (Hedera Helix), of Chinese thorowax (Bupleurum chinensis), of Bupleurum Falcatum, of arnica (Arnica Montana L), of rosemary (Rosmarinus officinalis N), of marigold (Calendula officinalis), of sage (Salvia officinalis L), of ginseng (Panax ginseng), of ginko biloba, of St.-John's-Wort (Hyperycum Perforatum), of butcher's-broom (Ruscus aculeatus L), of European meadowsweet (Filipendula ulmaria L), of big-flowered Jarva tea (Orthosiphon Stamincus Benth), of algae (Fucus Vesiculosus), of birch (Betula alba), of green tea, of cola nuts (Cola Nipida), of horse-chestnut, of bamboo, of spadeleaf (Centella asiatica), of heather, of fucus, of willow, of mouse-ear, of escine, of cangzhu, of chrysanthellum indicum, of the plants of the Armeniacea genus, Atractylodis Platicodon, Sinnomenum, Pharbitidis, Flemingia, of Coleus such as C. Forskohlii, C. blumei, C. esquirolii, C. scutellaroides, C. xanthantus and C. Barbatus, such as the extract of root of Coleus barbatus, extracts of Ballote, of Guioa, of Davallia, of Terminalia, of Barringtonia, of Trema, of antirobia, cecropia, argania, dioscoreae such as Dioscorea opposita or Mexican, extracts of Ammi visnaga, of Centella asiatica and Siegesbeckia, in particular Siegesbeckia orientalis, vegetable extracts of the family of Ericaceae, in particular bilberry extracts (Vaccinium angustifollium) or Arctostaphylos uva ursi, aloe vera, plant sterols (e.g., phytosterol), Manjistha (extracted from plants in the genus Rubia, particularly Rubia Cordifolia), and Guggal (extracted from plants in the genus Commiphora, particularly Commiphora Mukul), kola extract, chamomile, red clover extract, Piper methysticum extract (Kava Kava from SEDERMA (FR 2 771 002 and WO 99/25369), Bacopa monieri extract (Bacocalmine.TM. from SEDERMA, WO 99/40897) and sea whip extract, extracts of Glycyrrhiza glabra, of mulberry, of

melaleuca (tea tree), of Larrea divaricata, of Rabdosia rubescens, of euglena gracilis, of Fibraurea recisa Hirudinea, of Chaparral Sorghum, of sun flower extract, of Enantia chlorantha, of Mitracarpe of Spermacocea genus, of Buchu barosma, of Lawsonia inermis L., of Adiantium Capillus-Veneris L., of Chelidonium majus, of Luffa cylindrical, of Japanese Mandarin (Citrus reticulata Blanco var. unshiu), of Camelia sinensis, of Imperata cylindrical, of Glaucium Flavum, of Cupressus Sempervirens, of Polygonatum multiflorum, of loveyly hemsleya, of Sambucus Nigra, of Phaseolus lunatus, of Centaurium, of Macrocystis Pyrifera, of Turnera Diffusa, of Anemarrhena asphodeloides, of Portulaca pilosa, of Humulus lupulus, of Coffea Arabica and of Ilex Paraguariensis.

[0121] In one embodiment, the loaded exosomes can be combined with an extract of Portulaca pilosa. This plant also called "Kiss me quick" or "Amor Crescido" is part of the purslane family. It is a small edible oily plant rich in omega 3, vitamins A, B.sub.1, B.sub.2 and C and mucilage. It is used in cosmetics for its particular anti-inflammatory, soothing, softening and lightening properties and to treat hair (to give it volume and stimulate regrowth). Extraction from the plant may be performed using conventional techniques such as phenolic extraction, from any part of the plant such as the flower, seed, fruit, root, tubercle, leaf, pericarp and preferably rhizome. The extraction solvents may be selected from amongst water, propylene glycol, butylene glycol, glycerine, PEG-6 caprylic/capric glycerides, polyethylene glycol, methyl and/or ethyl esters, diglycols, cyclical polyols, ethoxylated or propoxylated diglycols, alcohols (methanol, ethanol, propanol, and butanol) or any mixture of these solvents. Plant extracts may also be obtained by other processes such as maceration, simple decoction, lixiviation, reflux extraction, super-critical extraction with CO2, ultrasound or microwave extraction or counter-current techniques. The excipient for the plant extract is selected in order to be compatible with the loaded exosomes and if necessary compatible with an excipient for the loaded exosomes with which the extract will subsequently be mixed and also to be compatible with the physical or galenic form of the final mixture which is to be obtained.

Additives

[0122] The cosmetic compositions may include various additional other ingredients, conventional or not. The decision to include an additional ingredient and the choice of a specific active ingredient and of additional ingredients depends on the specific application and product

formulation. Whether an ingredient is an "active" ingredient and an "additional" ingredient depends on the specific application and product type. A substance that is an "active" ingredient in one application or product may be a "functional" ingredient in another, and vice versa.

[0123] The cosmetic compositions may include one or more additional ingredients, various, conventional or not, which will provide some benefit to the object of the composition. Such additional ingredients may include one or more substances such as, without limitations, cleaning agents, hair conditioning agents, skin conditioning agents, hair styling agents, antidandruff agents, hair growth promoters, perfumes, sunscreen and/or sunblock compounds, pigments, moisturizers, film formers, hair colors, make-up agents, detergents, pharmaceuticals, thickening agents, emulsifiers, humectants, emollients, antiseptic agents, deodorant actives, surfactants and propellants.

[0124] In an embodiment, where the cosmetic composition is to be in contact with human keratinous tissue, the additional ingredients should be suitable for application to keratinous tissue, that is, when incorporated into the composition they are suitable for use in contact with human keratinous tissue (hair, nails, skin, lips) without undue toxicity, incompatibility, instability, allergic response.

[0125] The CTFA Cosmetic Ingredient Handbook, Tenth Edition (published by the Cosmetic, Toiletry, and Fragrance Association, Inc., Washington D.C.) (2004) describes a non-limited wide variety of cosmetic and pharmaceutical ingredients usually used in the skin care industry that can be used as additional ingredients in the compositions of the present invention. Examples of these ingredient classes include, but are not limited to: healing agents, skin anti-aging agents, skin moisturizing agents, anti-wrinkle agents, anti-atrophy agents, skin smoothing agents, antibacterial agents, antifungal agents, pesticides anti parasitic agents, antimicrobial agents, anti-inflammatory agents, anti-pruriginous agents, external anaesthetic agents, antiviral agents, keratolytic agents, free radicals scavengers, antiseborrheic agents, antidandruff agents, the agents modulating the differentiation, proliferation or pigmentation of the skin and agents accelerating penetration, desquamating agents, depigmenting or propigmenting agents, antiglycation agents, tightening agents, agents stimulating the synthesis of dermal or epidermal macromolecules and/or preventing their degradation; agents stimulating the proliferation of fibroblasts and/or keratinocytes or stimulating the differentiation of keratinocytes; muscle relaxants; antipollution

and/or anti-free radical agents; slimming agents, anticellulite agents, agents acting on the microcirculation; agents acting on the energy metabolism of the cells; cleaning agents, hair conditioning agents, hair styling agents, hair growth promoters, sunscreen and/or sunblock compounds, make-up agents, detergents, pharmaceutical drugs, emulsifiers, emollients, antiseptic agents, deodorant actives, dermatologically acceptable carriers, surfactants, abrasives, absorbents, aesthetic components such as fragrances, colorings/colorants, essential oils, skin sensates, cosmetic astringents, anti-acne agents, anti-caking agents, anti-foaming agents, antioxidants, binders, biological additives, enzymes, enzymatic inhibitors, enzyme-inducing agents, coenzymes, plant extracts, plant derivatives, plant tissue extracts, plant seed extracts, plant oils, botanicals, botanical extracts, ceramides, peptides, buffering agents, bulking agents, chelating agents, chemical additives, colorants, cosmetic biocides, denaturants, drug astringents, external analgesics, film formers or materials, e.g., polymers, for aiding the film-forming properties and substantivity of the composition, quaternary derivatives, agents increasing the substantivity, opacifying agents, pH adjusters, propellants, reducing agents, sequestrants, skin bleaching and lightening agents, skin tanning agents, skin-conditioning agents (e.g., humectants, including miscellaneous and occlusive), skin soothing and/or healing agents and derivatives, skin treating agents, thickeners, and vitamins and derivatives thereof, peeling agents, moisturizing agents, curative agents, lignans, preservatives, UV absorbers, a cytotoxic, an antineoplastic agent, a fat-soluble active, suspending agents, viscosity modifiers, dyes, nonvolatile solvents, diluents, pearlescent aids, foam boosters, a vaccine, and their mixture.

[0126] Moisturizing Agents

[0127] Non-limiting examples of moisturizing agents that can be used with the compositions of the present invention include amino acids, chondroitin sulfate, diglycerin, erythritol, fructose, glucose, glycerin, glycerol polymers, glycol, 1,2,6-hexanetriol, honey, hyaluronic acid, hydrogenated honey, hydrogenated starch hydrolysate, inositol, lactitol, maltitol, maltose, mannitol, natural moisturizing factor, PEG-15 butanediol, polyglyceryl sorbitol, salts of pyrollidone carboxylic acid, potassium PCA, propylene glycol, sodium glucuronate, sodium PCA, sorbitol, sucrose, trehalose, urea, and xylitol.

[0128] Other examples include acetylated lanolin, acetylated lanolin alcohol, alanine, algae extract, aloe barbadensis, aloe-barbadensis extract, aloe barbadensis gel, althea officinalis

extract, apricot (prunus armeniaca) kernel oil, arginine, arginine aspartate, arnica montana extract, aspartic acid, avocado (persea gratissima) oil, barrier sphingolipids, butyl alcohol, beeswax, behenyl alcohol, beta-sitosterol, birch (betula alba) bark extract, borage (borago officinalis) extract, butcherbroom (ruscus aculeatus) extract, butylene glycol, calendula officinalis extract, calendula officinalis oil, candelilla (euphorbia cerifera) wax, canola oil, caprylic/capric triglyceride, cardamon (elettaria cardamomum) oil, carnauba (copernicia cerifera) wax, carrot (daucus carota sativa) oil, castor (ricinus communis) oil, ceramides, ceresin, ceteareth-5, ceteareth-12, ceteareth-20, cetearyl octanoate, ceteth-20, ceteth-24, cetyl acetate, cetyl octanoate, cetyl palmitate, chamomile (anthemis nobilis) oil, cholesterol, cholesterol esters, cholesteryl hydroxystearate, citric acid, clary (salvia sclarea) oil, cocoa (theobroma cacao) butter, coco-caprylate/caprate, coconut (cocos nucifera) oil, collagen, collagen amino acids, corn (zea mays) oil, fatty acids, decyl oleate, dimethicone copolyol, dimethiconol, dioctyl adipate, dioctyl succinate, dipentaerythrityl hexacaprylate/hexacaprate, DNA, erythritol, ethoxydiglycol, ethyl linoleate, eucalyptus globulus oil, evening primrose (oenothera biennis) oil, fatty acids, geranium maculatum oil, glucosamine, glucose glutamate, glutamic acid, glycereth-26, glycerin, glycerol, glyceryl distearate, glyceryl hydroxystearate, glyceryl laurate, glyceryl linoleate, glyceryl myristate, glyceryl oleate, glyceryl stearate, glyceryl stearate SE, glycine, glycol stearate, glycol stearate SE, glycosaminoglycans, grape (vitis vinifera) seed oil, hazel (corylus americana) nut oil, hazel (corylus avellana) nut oil, hexylene glycol, hyaluronic acid, hybrid safflower (carthamus tinctorius) oil, hydrogenated castor oil, hydrogenated coco-glycerides, hydrogenated coconut oil, hydrogenated lanolin, hydrogenated lecithin, hydrogenated palm glyceride, hydrogenated palm kernel oil, hydrogenated soybean oil, hydrogenated tallow glyceride, hydrogenated vegetable oil, hydrolyzed collagen, hydrolyzed elastin, hydrolyzed glycosaminoglycans, hydrolyzed keratin, hydrolyzed soy protein, hydroxylated lanolin, hydroxyproline, isocetyl stearate, isocetyl stearoyl stearate, isodecyl oleate, isopropyl isostearate, isopropyl lanolate, isopropyl myristate, isopropyl palmitate, isopropyl stearate, isostearamide DEA, isostearic acid, isostearyl lactate, isostearyl neopentanoate, jasmine (jasminum officinale) oil, jojoba (buxus chinensis) oil, kelp, kukui (aleurites moluccana) nut oil, lactamide MEA, laneth-16, laneth-10 acetate, lanolin, lanolin acid, lanolin alcohol, lanolin oil, lanolin wax, lavender (lavandula angustifolia) oil, lecithin, lemon (citrus medica limonum) oil, linoleic acid, linolenic acid, macadamia ternifolia nut oil, maltitol, matricaria (chamomilla recutita) oil, methyl

glucose sesquistearate, methylsilanol PCA, mineral oil, mink oil, mortierella oil, myristyl lactate, myristyl myristate, myristyl propionate, neopentyl glycol dicaprylate/dicaprate, octyldodecanol, octyldodecyl myristate, octyldodecyl stearoyl stearate, octyl hydroxystearate, octyl palmitate, octyl salicylate, octyl stearate, oleic acid, olive (olea europaea) oil, orange (citrus aurantium dulcis) oil, palm (elaeis guineensis) oil, palmitic acid, pantethine, panthenol, panthenyl ethyl ether, paraffin, PCA, peach (prunus persica) kernel oil, peanut (arachis hypogaea) oil, PEG-8 C12-18 ester, PEG-15 cocamine, PEG-150 distearate, PEG-60 glyceryl isostearate, PEG-5 glyceryl stearate, PEG-30 glyceryl stearate, PEG-7 hydrogenated castor oil, PEG-40 hydrogenated castor oil, PEG-60 hydrogenated castor oil, PEG-20 methyl glucose sesquistearate, PEG40 sorbitan peroleate, PEG-5 soy sterol, PEG-10 soy sterol, PEG-2 stearate, PEG-8 stearate, PEG-20 stearate, PEG-32 stearate, PEG40 stearate, PEG-50 stearate, PEG-100 stearate, PEG-150 stearate, pentadecalactone, peppermint (mentha piperita) oil, petrolatum, phospholipids, polyamino sugar condensate, polyglyceryl-3 diisostearate, polyquaternium-24, polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, polysorbate 85, Zemea (propanediol), potassium myristate, potassium palmitate, propylene glycol, propylene glycol dicaprylate/dicaprate, propylene glycol dioctanoate, propylene glycol dipelargonate, propylene glycol laurate, propylene glycol stearate, propylene glycol stearate SE, PVP, PVP K-30, pyridoxine dipalmitate, retinol, retinyl palmitate, rice (oryza sativa) bran oil, RNA, rosemary (rosmarinus officinalis) oil, rose oil, safflower (carthamus tinctorius) oil, sage (salvia officinalis) oil, sandalwood (santalum album) oil, serine, serum protein, sesame (sesamum indicum) oil, shea butter (butyrospermum parkii), silk powder, sodium chondroitin sulfate, sodium hyaluronate, sodium lactate, sodium palmitate, sodium PCA, sodium polyglutamate, soluble collagen, sorbitan laurate, sorbitan oleate, sorbitan palmitate, sorbitan sesquioleate, sorbitan stearate, sorbitol, soybean (glycine soja) oil, sphingolipids, squalane, squalene, stearamide MEA-stearate, stearic acid, stearoxy dimethicone, stearoxytrimethylsilane, stearyl alcohol, stearyl glycyrrhetinate, stearyl heptanoate, stearyl stearate, sunflower (helianthus annuus) seed oil, sweet almond (prunus amygdalus dulcis) oil, synthetic beeswax, tocopherol, tocopheryl acetate, tocopheryl linoleate, tribehenin, tridecyl neopentanoate, tridecyl stearate, triethanolamine, tristearin, urea, vegetable oil, water, waxes, wheat (triticum vulgare) germ oil, and ylang ylang (cananga odorata) oil.

[0129] Antioxidants

[0130] Non-limiting examples of antioxidants that can be used with the compositions of the present invention include acetyl cysteine, ascorbic acid polypeptide, ascorbyl dipalmitate, ascorbyl methylsilanol pectinate, ascorbyl palmitate, ascorbyl stearate, BHA, BHT, t-butyl hydroquinone, cysteine, cysteine HCl, diamylhydroquinone, di-t-butylhydroquinone, dicetyl thiodipropionate, dioleyl tocopheryl methylsilanol, disodium ascorbyl sulfate, distearyl thiodipropionate, ditridecyl thiodipropionate, dodecyl gallate, erythorbic acid, esters of ascorbic acid, ethyl ferulate, ferulic acid, gallic acid esters, hydroquinone, isooctyl thioglycolate, kojic acid, magnesium ascorbate, magnesium ascorbyl phosphate, methylsilanol ascorbate, natural botanical anti-oxidants such as green tea or grape seed extracts, nordihydroguaiaretic acid, octyl gallate, phenylthioglycolic acid, potassium ascorbyl tocopheryl phosphate, potassium sulfite, propyl gallate, quinones, rosmarinic acid, sodium ascorbate, sodium bisulfite, sodium erythorbate, sodium metabisulfite, sodium sulfite, superoxide dismutase, sodium thioglycolate, sorbityl furfural, thiodiglycol, thiodiglycolamide, thiodiglycolic acid, thioglycolic acid, thiolactic acid, thiosalicylic acid, tocophereth-5, tocophereth-10, tocophereth-12, tocophereth-18, tocophereth-50, tocopherol, tocophersolan, tocopheryl acetate, tocopheryl linoleate, tocopheryl nicotinate, tocopheryl succinate, and tris(nonylphenyl)phosphite.

[0131] Structuring Agents

[0132] In other non-limiting aspects, the compositions of the present invention can include a structuring agent. Structuring agent, in certain aspects, assist in providing rheological characteristics to the composition to contribute to the composition's stability. In other aspects, structuring agents can also function as an emulsifier or surfactant. Non-limiting examples of structuring agents include stearic acid, palmitic acid, stearyl alcohol, cetyl alcohol, behenyl alcohol, stearic acid, palmitic acid, the polyethylene glycol ether of stearyl alcohol having an average of about 1 to about 21 ethylene oxide units, the polyethylene glycol ether of cetyl alcohol having an average of about 1 to about 5 ethylene oxide units, and mixtures thereof.

[0133] Preservative

[0134] Non-limiting examples of preservatives that can be used in the context of the present invention include quaternary ammonium preservatives such as polyquaternium-1 and benzalkonium halides (e.g., benzalkonium chloride ("BAC") and benzalkonium bromide),

parabens (e.g., methylparabens and propylparabens), phenoxyethanol, benzyl alcohol, chlorobutanol, phenol, sorbic acid, thimerosal or combinations thereof. Preservatives may also include methyparaben, propylparaben, butylparaben, ethylparaben, and isobutylparaben, and any combination thereof. An exemplary preservative can be Mikrokill COS.

[0135] Emulsifiers

[0136] Emulsifiers can reduce the interfacial tension between phases and improve the formulation and stability of an emulsion. The emulsifiers can be nonionic, cationic, anionic, and zwitterionic emulsifiers (See McCutcheon's (1986); U.S. Pat. Nos. 5,011,681; 4,421,769; 3,755,560). Non-limiting examples include esters of glycerin, esters of propylene glycol, fatty acid esters of polyethylene glycol, fatty acid esters of polypropylene glycol, esters of sorbitol, esters of sorbitan anhydrides, carboxylic acid copolymers, esters and ethers of glucose, ethoxylated ethers, ethoxylated alcohols, alkyl phosphates, polyoxyethylene fatty ether phosphates, fatty acid amides, acyl lactylates, soaps, TEA stearate, DEA oleth-3 phosphate, polyethylene glycol 20 sorbitan monolaurate (polysorbate 20), polyethylene glycol 5 soya sterol, steareth-2, steareth-20, steareth-21, ceteareth-20, PPG-2 methyl glucose ether distearate, ceteth-10, polysorbate 80, cetyl phosphate, potassium cetyl phosphate, diethanolamine cetyl phosphate, polysorbate 60, glyceryl stearate, PEG-100 stearate, and mixtures thereof. An emulsifier can also be Lipowax D, or Arlacel 165. Emulsifiers can be in the range of 1-5%, 2-5%, 3-7% of the composition.

[0137] Thickening Agents

[0138] Thickening agents, including thickener or gelling agents, include substances which that can increase the viscosity of a composition. Thickeners include those that can increase the viscosity of a composition without substantially modifying the efficacy of the active ingredient within the composition. Thickeners can also increase the stability of the compositions of the present invention. In certain aspects of the present invention, thickeners include hydrogenated polyisobutene or trihydroxystearin, or a mixture of both.

[0139] Non-limiting examples of additional thickening agents that can be used in the context of the present invention include carboxylic acid polymers, crosslinked polyacrylate polymers, polyacrylamide polymers, polysaccharides, and gums. Examples of carboxylic acid polymers

include crosslinked compounds containing one or more monomers derived from acrylic acid, substituted acrylic acids, and salts and esters of these acrylic acids and the substituted acrylic acids, wherein the crosslinking agent contains two or more carbon-carbon double bonds and is derived from a polyhydric alcohol (see U.S. Pat. Nos. 5,087,445; 4,509,949; 2,798,053; CTFA International Cosmetic Ingredient Dictionary, Fourth edition, 1991, pp. 12 and 80). Examples of commercially available carboxylic acid polymers include carbomers, which are homopolymers of acrylic acid crosslinked with allyl ethers of sucrose or pentaerytritol (e.g., Carbopol™ 900 or 980 series from B. F. Goodrich).

[0140] Non-limiting examples of crosslinked polyacrylate polymers include cationic and nonionic polymers. Examples are described in U.S. Pat. Nos. 5,100,660; 4,849,484; 4,835,206; 4,628,078; 4,599,379).

[0141] Non-limiting examples of polyacrylamide polymers (including nonionic polyacrylamide polymers including substituted branched or unbranched polymers) include polyacrylamide, isoparaffin and laureth-7, multi-block copolymers of acrylamides and substituted acrylamides with acrylic acids and substituted acrylic acids.

[0142] Non-limiting examples of polysaccharides include cellulose, carboxymethyl hydroxyethylcellulose, cellulose acetate propionate carboxylate, hydroxyethylcellulose, hydroxyethyl ethylcellulose, hydroxypropylcellulose, hydroxypropyl methylcellulose, methyl hydroxyethylcellulose, microcrystalline cellulose, sodium cellulose sulfate, and mixtures thereof. Another example is an alkyl substituted cellulose where the hydroxy groups of the cellulose polymer is hydroxyalkylated (preferably hydroxy ethylated or hydroxypropylated) to form a hydroxyalkylated cellulose which is then further modified with a C.sub.10-C.sub.30 straight chain or branched chain alkyl group through an ether linkage. Typically these polymers are ethers of C.sub.10-C.sub.30 straight or branched chain alcohols with hydroxyalkylcelluloses. Other useful polysaccharides include scleroglucans comprising a linear chain of (1-3) linked glucose units with a (1-6) linked glucose every three unit.

[0143] Non-limiting examples of gums that can be used with the present invention include acacia, agar, algin, alginic acid, ammonium alginate, amylopectin, calcium alginate, calcium carrageenan, carnitine, carrageenan, dextrin, gelatin, gellan gum, guar gum, guar

hydroxypropyltrimonium chloride, hectorite, hyaluroinic acid, hydrated silica, hydroxypropyl chitosan, hydroxypropyl guar, karaya gum, kelp, locust bean gum, natto gum, potassium alginate, potassium carrageenan, propylene glycol alginate, sclerotium gum, sodium carboyxmethyl dextran, sodium carrageenan, tragacanth gum, xanthan gum, and mixtures thereof.

[0144] Silicone Containing Compounds

[0145] In non-limiting aspects, silicone containing compounds include any member of a family of polymeric products whose molecular backbone is made up of alternating silicon and oxygen atoms with side groups attached to the silicon atoms. By varying the --Si--O-- chain lengths, side groups, and crosslinking, silicones can be synthesized into a wide variety of materials. They can vary in consistency from liquid to gel to solids.

[0146] The silicone containing compounds that can be used in the context of the present invention include those described in this specification or those known to a person of ordinary skill in the art. Non-limiting examples include silicone oils (e.g., volatile and non-volatile oils), gels, and solids. In certain aspects, the silicon containing compounds includes a silicone oils such as a polyorganosiloxane. Non-limiting examples of polyorganosiloxanes include dimethicone, cyclomethicone, polysilicone-11, phenyl trimethicone, trimethylsilylamodimethicone, stearoxytrimethylsilane, or mixtures of these and other organosiloxane materials in any given ratio in order to achieve the desired consistency and application characteristics depending upon the intended application (e.g., to a particular area such as the skin, hair, or eyes). A "volatile silicone oil" includes a silicone oil have a low heat of vaporization, i.e. normally less than about 50 cal per gram of silicone oil. Non-limiting examples of volatile silicone oils include: cyclomethicones such as Dow Corning 344 Fluid, Dow Corning 345 Fluid, Dow Corning 244 Fluid, and Dow Corning 245 Fluid, Volatile Silicon 7207 (Union Carbide Corp., Danbury, Conn.); low viscosity dimethicones, i.e. dimethicones having a viscosity of about 50 cst or less (e.g., dimethicones such as Dow Corning 200-0.5 cst Fluid). The Dow Corning Fluids are available from Dow Corning Corporation, Midland, Mich. Cyclomethicone and dimethicone are described in the Third Edition of the CTFA Cosmetic Ingredient Dictionary (incorporated by reference) as cyclic dimethyl polysiloxane compounds and a mixture of fully methylated linear siloxane polymers end-blocked with trimethylsiloxy units, respectively. Other non-limiting

volatile silicone oils that can be used in the context of the present invention include those available from General Electric Co., Silicone Products Div., Waterford, N.Y. and SWS Silicones Div. of Stauffer Chemical Co., Adrian, Mich.

[0147] Essential Oils

[0148] Essential oils include oils derived from herbs, flowers, trees, and other plants. Such oils are typically present as tiny droplets between the plant's cells, and can be extracted by several method known to those of skill in the art (e.g., steam distilled, enfleurage (i.e., extraction by using fat), maceration, solvent extraction, or mechanical pressing). When these types of oils are exposed to air they tend to evaporate (i.e., a volatile oil). As a result, many essential oils are colorless, but with age they can oxidize and become darker. Essential oils are insoluble in water and are soluble in alcohol, ether, fixed oils (vegetal), and other organic solvents. Typical physical characteristics found in essential oils include boiling points that vary from about 160° to 240°C and densities ranging from about 0.759 to about 1.096.

[0149] Essential oils typically are named by the plant from which the oil is found. For example, rose oil or peppermint oil are derived from rose or peppermint plants, respectively. Non-limiting examples of essential oils that can be used in the context of the present invention include sesame oil, macadamia nut oil, tea tree oil, evening primrose oil, Spanish sage oil, Spanish rosemary oil, coriander oil, thyme oil, pimento berries oil, rose oil, anise oil, balsam oil, bergamot oil, rosewood oil, cedar oil, chamomile oil, sage oil, clary sage oil, clove oil, cypress oil, eucalyptus oil, fennel oil, sea fennel oil, frankincense oil, geranium oil, ginger oil, grapefruit oil, jasmine oil, juniper oil, lavender oil, lemon oil, lemongrass oil, lime oil, mandarin oil, marjoram oil, myrrh oil, neroli oil, orange oil, patchouli oil, pepper oil, black pepper oil, petitgrain oil, pine oil, rose otto oil, rosemary oil, sandalwood oil, spearmint oil, spikenard oil, vetiver oil, wintergreen oil, or ylang ylang. Other essential oils known to those of skill in the art are also contemplated as being useful within the context of the present invention.

[0150] Other additional ingredients may include sugar amines, glucosamine, D-glucosamine, N-acetyl glucosamine, N-acetyl-D-glucosamine, mannosamine, N-acetyl mannosamine, galactosamine, N-acetyl galactosamine, vitamin B3 and its derivatives, niacinamide, sodium dehydroacetate, dehydroacetic acid and its salts, phytosterols, salicylic acid compounds,

hexamidines, dialkanoyl hydroxyproline compounds, soy extracts and derivatives, equol, isoflavones, flavonoids, phytantriol, farnesol, geraniol, peptides and their derivatives, di-, tri-, tetra-, penta-, and hexapeptides and their derivatives, lys-thr-thr-lys-ser, palmitoyl-lys-thr-thr-lys-ser, carnosine, N-acyl amino acid compounds, retinoids, retinyl propionate, retinol, retinyl palmitate, retinyl acetate, retinal, retinoic acid, water-soluble vitamins, ascorbates, vitamin C, ascorbic acid, ascorbyl glucoside, ascorbyl palmitate, magnesium ascorbyl phosphate, sodium ascorbyl phosphate, vitamins their salts and derivatives, provitamins and their salts and derivatives, ethyl panthenol, vitamin B, vitamin B derivatives, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin K, vitamin K derivatives, pantothenic acid and its derivatives, pantothenyl ethyl ether, panthenol and its derivatives, dexpanthenol, biotin, amino acids and their salts and derivatives, water soluble amino acids, asparagine, alanine, indole, glutamic acid, water insoluble vitamins, vitamin A, vitamin E, vitamin F, vitamin D, mono-, di-, and tri-terpenoids, beta-ionol, cedrol, and their derivatives, water insoluble amino acids, tyrosine, tryptamine, butylated hydroxytoluene, butylated hydroxyanisole, allantoin, tocopherol nicotinate, tocopherol, tocopherol esters, palmitoyl-gly-his-lys, phytosterol, hydroxy acids, glycolic acid, lactic acid, lactobionic acid, keto acids, pyruvic acid, phytic acid, lysophosphatidic acid, stilbenes, cinnamates, resveratrol, kinetin, zeatin, dimethylaminoethanol, natural peptides, soy peptides, salts of sugar acids, Mn gluconate, Zn gluconate, particulate materials, pigment materials, natural colors, piroctone olamine, 3,4,4'-trichlorocarbanilide, triclocarban, zinc pyrithione, hydroquinone, kojic acid, ascorbic acid, magnesium ascorbyl phosphate, ascorbyl glucoside, pyridoxine, aloe vera, terpene alcohols, allantoin, bisabolol, dipotassium glycyrrhizinate, glycerol acid, sorbitol acid, pentaerythritol acid, pyrrolidone acid and its salts, dihydroxyacetone, erythrulose, glyceraldehyde, tartaraldehyde, clove oil, menthol, camphor, eucalyptus oil, eugenol, menthyl lactate, witch hazel distillate, eicosene and vinyl pyrrolidone copolymers, iodopropyl butylcarbamate, a polysaccharide, an essential fatty acid, salicylate, glycyrrhetinic acid, carotenoides, ceramides and pseudo-ceramides, a lipid complex, oils in general of natural origin such shea butter, apricot oil, onagre oil, prunus oil, palm oil, monoi oil, HEPES; procysteine; O-octanoyl-6-D-maltose; the disodium salt of methylglycinediacetic acid, steroids such as diosgenin and derivatives of DHEA; DHEA or dehydroepiandrosterone and/or a precursor or chemical or biological derivative, N-ethyloxycarbonyl-4-para-aminophenol, bilberry extracts; phytohormones; extracts of the yeast Saccharomyces cerevisiae; extracts of algae;

extracts of soyabean, lupin, maize and/or pea; alverine and its salts, in particular alverine citrate, extract of butcher's broom and of horse chestnut, and mixtures thereof, a metallopreoteinase inhibitor.

Methods of Use

[0151] In one embodiment, a method of treating skin can include firming skin or reducing the appearance of fine lines or wrinkles comprising topically applying any one of the compositions of the present invention to skin in need of such treatment. The composition can include an exosome loaded with at least a skin care peptide or combinations thereof and a dermatologically acceptable vehicle. The composition can be applied to fine lines or wrinkles, sagging skin, skin areas prone to sagging, etc.

[0152] The composition can be applied to all types of skin such as the face, neck, decolette, arms, hands, body, legs, feet, etc. The composition can be formulated as a cream, lotion, gel, gel-like cream, serum, etc. The color of the composition can be opaque, transparent, translucent, etc.

[0153] The method can further comprise identifying a person in need of skin treatment. The person can be a male or female. The age of the person can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or more years old, or any range derivable therein.

[0154] Also disclosed is a method of treating or preventing a skin condition comprising topically applying any composition disclosed throughout the specification and claims to skin having a skin condition or at risk of having a skin condition, wherein topical application of the composition to the skin condition treats the skin condition or prevents the skin condition from forming. In particular embodiments, the skin condition is a fine line or wrinkle, dry or flaky skin, erythema, sensitive skin, or inflamed skin. In particular aspects, erythema, sensitive skin, or inflamed skin is caused by skin sunburn, electrical treatments of skin, skin burns, contact allergies, systemic allergies, skin toxicity, exercise, insect stings, bacterial infection, viral infection, fungal infection, protozoa infection, massage, or windburn. In other aspects, the following additional skin conditions can be treated or prevented in accordance with the methods and compositions disclosed throughout the specification and claims: pruritus, spider veins, lentigo, age spots, senile purpura, keratosis, melasma, blotches, nodules, sun damaged skin, dermatitis (including, but not

limited to seborrheic dermatitis, nummular dermatitis, contact dermatitis, atopic dermatitis, exfoliative dermatitis, perioral dermatitis, and stasis dermatitis), psoriasis, folliculitis, rosacea, acne, impetigo, erysipelas, erythrasma, eczema, and other inflammatory skin conditions. In certain non-limiting aspects, the skin condition can be caused by exposure to UV light, age, irradiation, chronic sun exposure, environmental pollutants, air pollution, wind, cold, heat, chemicals, disease pathologies, smoking, or lack of nutrition. The skin can be facial skin or non-facial skin (e.g., arms, legs, hands, chest, back, feet, etc.). The method can further comprise identifying a person in need of skin treatment. The person can be a male or female. The age of the person can be at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or more years old, or any range derivable therein. The method can also include topically applying an amount effective to: increase the stratum corneum turnover rate of the skin; increase collagen synthesis in fibroblasts; increase cellular anti-oxidant defense mechanisms (e.g., exogenous additions of anti-oxidants can bolster, replenish, or prevent the loss of cellular antioxidants such as catalase and glutathione in skin cells (e.g., keratinocytes, melanocytes, langerhans cells, etc.) which will reduce or prevent oxidative damage to the skin, cellular, proteins, and lipids); inhibit melanin production in melanocytes; reduce or prevent oxidative damage to skin (including reducing the amount lipid peroxides and/or protein oxidation in the skin). In one instance, the composition includes palmitoyl tetrapeptide-7, methylsilanol mannuronate, Lactobacillus ferment, and plant extracts from Punica granatum, Castanea sativa, Gossypium hirsutum, and Euterpe oleracea, which can treat a wide range of skin conditions.

[0155] Also disclosed is a method of reducing the appearance of uneven skin tone comprising topically applying any one of the compositions disclosed throughout the specification and claims to skin having an uneven skin tone, wherein topical application of the composition to uneven skin tone reduces the appearance of uneven skin tone. In one instance, the composition includes palmitoyl tetrapeptide-7, methylsilanol mannuronate, Lactobacillus ferment, and plant extracts from Punica granatum, Castanea sativa, Gossypium hirsutum, and Euterpe oleracea.

[0156] In another embodiment, there is disclosed a method of reducing pain associated with erythema, sensitive skin, or inflamed skin, comprising topically applying any one of the compositions disclosed throughout the specification and claims to erythemic, sensitive, or inflamed skin, wherein topical application of the composition to erythemic, sensitive, or

inflamed skin reduces the pain associated with erythema, sensitive skin, or inflamed skin. In one instance, the composition includes palmitoyl tetrapeptide-7, methylsilanol mannuronate, Lactobacillus ferment, and plant extracts from Punica granatum, Castanea sativa, Gossypium hirsutum, and Euterpe oleracea.

[0157] In still another aspect, there is disclosed a method of reducing the appearance of symptoms associated with erythema, sensitive skin, or inflamed skin, comprising topically applying any one of the compositions disclosed throughout the specification and claims erythemic, sensitive, or inflamed skin, wherein topical application of the composition to erythemic, sensitive, or inflamed skin reduces the appearance of symptoms associated with erythema, sensitive skin, or inflamed skin. In one instance, the composition includes palmitoyl tetrapeptide-7, methylsilanol mannuronate, Lactobacillus ferment, and plant extracts from Punica granatum, Castanea sativa, Gossypium hirsutum, and Euterpe oleracea.

[0158] Also disclosed is a method of removing dirt, oil, or make-up from skin comprising: applying any one of the compositions disclosed throughout the specification and claims to skin in need of removal of dirt, oil, or make-up; and removing the composition from the skin with water within about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20, 30, 40, 50, 60, 120, 180, or minutes after application, wherein dirt, oil, or makeup is removed from the skin. In particular aspects, the composition is applied to dirt on the skin, and wherein the dirt is removed from the skin. In other aspects, the composition is applied to oil on the skin, and wherein the oil is removed from the skin. In another embodiment, the composition is applied to make-up on the skin, and wherein the make-up is removed from the skin.

[0159] In other aspect, there is disclosed a method of increasing collagen production in a skin cell comprising topically applying any one of the compositions disclosed throughout the specification and claims to a skin cell in need of collagen production, wherein the topical application of the composition to the skin cell increases collagen production in the skin cell. Non-limiting examples of such cells include human epidermal keratinocyte, human fibroblast dermal cell, human melanocytes, three dimensional human cell-derived in vitro tissue equivalents comprising human keratinocytes, human fibroblasts, or human melanocytes, or any combination thereof (e.g., combination of human keratinocytes and human fibroblasts or a combination of human keratinocytes and human melanocytes).

[0160] Also provided is a method of treating skin during sleep. The method can include topically applying a composition to a user's skin prior to the user falling asleep. The composition can include milk-derived exosomes loaded with at least on skin care peptide or a combination therein as described and a dermatologically acceptable vehicle, a vehicle which can have hydrating and/or moisturization properties. The method can further include removing the composition from the user's skin after the user wakes up. This can result in rejuvenation or replenishing the skin during the evening hours by using the unique combination of ingredients. This combination is proven to be chemically compatible (i.e., they are able to coexist together without detrimentally affecting their individual skin efficacy abilities) and skin friendly in that the combination does not appear to irritate the skin. The result of this combination is a superior way to rejuvenate the skin during the evening hours, which ultimately provides for an effective way to treat a wide variety of skin conditions such as firming/toning the skin, increasing the skin's elasticity, reducing the appearance of dark spots or aged spots, evening out the skin's tone, reducing the appearance of fine lines and wrinkles, reducing other signs of premature skin aging, and reducing the appearance of expression lines. The evening hours typically includes the time the sun goes down to the time the sun comes up. The composition can be applied during the evening hours prior to falling asleep and can be removed when the user awakens. The composition can be formulated for use on dry skin, normal skin, oily skin, combination skin, etc. The dermatologically acceptable vehicle can be formulated to moisturize skin, hydrate skin, to provide a substantive effect in that the composition has the ability to remain on the skin even during sleep mode.

Kits

[0161] Kits are also contemplated as being used in certain aspects of the present invention. For instance, compositions of the present invention can be included in a kit. A kit can include a container. Containers can include a bottle, a metal tube, a laminate tube, a plastic tube, a dispenser, a pressurized container, a barrier container, a package, a compartment, a lipstick container, a compact container, cosmetic pans that can hold cosmetic compositions, or other types of containers such as injection or blow-molded plastic containers into which the dispersions or compositions or desired bottles, dispensers, or packages are retained. The kit

and/or container can include indicia on its surface. The indicia, for example, can be a word, a phrase, an abbreviation, a picture, or a symbol.

[0162] The containers can dispense a pre-determined amount of the composition. In other embodiments, the container can be squeezed (e.g., metal, laminate, or plastic tube) to dispense a desired amount of the composition. The composition can be dispensed as a spray, an aerosol, a liquid, a fluid, or a semi-solid. The containers can have spray, pump, or squeeze mechanisms. A kit can also include instructions for employing the kit components as well the use of any other compositions included in the container. Instructions can include an explanation of how to apply, use, and maintain the compositions.

EXAMPLES

[0163] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

[0164] Example 1. Isolation of exosomes from bovine milk

[0165] Fresh bovine milk was obtained and milk fat globules, casein aggregate and debris was removed from the milk through centrifugation at 13,000 xg at 4°C for 30 min. Remaining milk fat globules were removed by straining the supernatant. Further removal of casein, large particles and microvesicle removal was performed by centrifugation at 100,000xg for 60 min at 4°C.

[0166] The supernatant (exo fraction) was isolated and transferred to a separate tube for further wash. Microvesicles were removed from the exo fraction by filtering the exo fraction sequentially through filters that were 10μm -> 1μm -> 0.45μm -> 0.22μm. Casein from the milk was removed via casein precipitation using 1M acetic acid. The precipitated casein was then removed by centrifugation. Exosome supernatant was then filtered through a 0.22μm filter to remove remaining particles. The pH of the exo fraction was restored using 1M sodium carbonate solution and filtered through a 0.22μm steri-cup before storage at 4°C. Milk exosomes were further purified from milk proteins using size exclusion chromatography using iZon columns.

[0167] Results: As demonstrated in Figs 1A-C, the isolated milk exosomes had the following characteristics: (1) rounded particles with a size range of 50-150nm observed as measured by

TEM (Transmission Electron Microscopy), proper size distribution as measured by NanoSight and expressed exosome markers such as CD9, CD63 and CD81.

[0168] Example 2: Methods of Loading Milk Exosomes with Various Skin Peptides

[0169] Various techniques were tested to load exosomes with tagged (FLAG-Biotin) and untagged skin peptides such as Pal-Tri-38, Pal-Tri-1, Pal-Tetra-7, Tetra-7. Exosomes were loaded using methods such as sonication, freeze/thaw, extrusion, or coincubation with PBS,. In all, 172 conditions were tested (including varying concentrations of peptides and exosomes, healing time, temperature, incubation time, etc).

[0170] Freeze thaw: Peptides were loaded onto milk exosomes with 4 cycles of freeze/thaw. Healing times were varied from 1 hour to O/N.

[0171] Sonication: 60% power, 20 x 20 sec pulses, O/N healing.

[0172] Extrusion :0.1um membrane was utilized with O/N healing.

[0173] Coincubation (O/N)

[0174] Treated samples were washed with size exclusion columns (iZon) and exosome fractions 6-8 were collected and combined. Samples were then lysed with either 1% SDS at room temperature overnight (HPLC) or with 1% Triton-X for 1 hour at 95°C (ELISA). Samples were analyzed for loading efficiency via HPLC (non-FLAG peptides) or ELISA (FLAG-peptides).

[0175] For all experiments, donor variability and reproducibility was checked. 3 separate bovine milk lots were used to isolate exosomes. 4 different exosome: skin peptide concentrations were tested.

[0176] Results:

[0177] Table 1: Max efficiency of loading exosomes via various methods as measured by HPLC

Method	Maximum Efficiency (%)				
	Pal-Tetra7	Pal-Tri38	Pal-Tri1	Pal-Penta4	Pal-Hexa12
Co-incubation	**62.74%**	**59.26%**	**79.23%**	73.90%	73.80%

Extrusion	41.78%	49.01%	57.98%	39.95%	68.44%
Freeze-thaw	51.36%	50.84%	70.29%	74.30%	69.08%
Sonication	46.10%	59.06%	68.29%	68.57%	72.60%
Maximum	62.74%	59.26%	79.23%	74.30%	73.80%

Table 2: Average efficiency of loading exosomes via various as measured by HPLC

Method	Average Efficiency (%)				
	Pal-Tetra7	Pal-Tri38	Pal-Tri1	Pal-Penta4	Pal-Hexa12
Co-incubation	30.70%	39.58%	51.45%	43.22%	50.96%
Extrusion	30.36%	35.36%	36.52%	21.40%	41.03%
Freeze-thaw	34.30%	39.49%	45.20%	39.24%	45.34%
Sonication	34.55%	42.07%	45.72%	44.49%	64.72%
Average	32.48%	39.13%	44.72%	37.09%	50.51%

[0178] Example 3 Bioactivity

[0179] 2D Bioactivity

[0180] Pal-Tri-38 was loaded into milk exosome with freeze/thaw. The fibroblast cells or keratinocytes were treated with either 1μM peptide only or loaded exosome for 72 hours and then the cells were collected and followed by total RNA isolation and qRT-PCR. The expression (fold increase) of collagen I, elastin and fibronectin in peptide only or loaded exosomes were compared with media alone and graphed.

[0181] Results:

[0182] Fibroblasts: As shown in Figure 2A-C, 8 of 10 fibroblast donors showed up-regulation of Collagen I (COL1) with treatment of loaded exosome (Exo Loaded) compared with peptide only sample. 2 of 10 showed moderate upregulation. 7 of 10 fibroblast donors showed up-regulation of Elastin (ELN), 2 of 10 showed moderate upregulation. 1 of 10 showed no/negative response. 8 of 10 fibroblast donors showed up-regulation of Fibronectin (FN) and 2 of 10 showed moderate response.

[0183] Keratinocyte: As shown in Figure 2D-E, 3 of 3 keratinocytes donors showed up-regulation of Collagen I (COL1) with treatment of loaded exosome (Exo Loaded) compared with peptide only sample. 3 of 3 Keratinocyte donors showed up-regulation of Fibronectin (FN).

[0184] Pal-Tri-1 was loaded into milk exosome using freeze/thaw methods. The fibroblast cells or keratinocytes were treated with either peptide only or loaded exosome for 72 hours and then the cells were collected and followed by total RNA isolation and qRT-PCR. The expression (fold increase) of collagen I, elastin and fibronectin in peptide only or loaded exosomes were compared with media alone and graphed.

[0185] Fibroblasts: As shown in Figure 3A-C, 8 of 8 fibroblast donors showed up-regulation of Collagen I (COL1) with treatment of loaded exosome (Exo Loaded) compared with peptide only sample. 4 of 8 fibroblast donors showed up-regulation of Elastin (ELN), 3 of 8 gave moderate up-regulation. 1 of 8 showed no/negative response. 8 of 8 fibroblast donors showed up-regulation of Fibronectin (FN)

[0186] Keratinocyte: As shown in Figure 3D-E, 6 of 6 keratinocytes donors showed up-regulation of Collagen I (COL1) with treatment of loaded exosome (Exo Loaded) compared with peptide only sample. 6 of 6 Keratinocyte donors showed up-regulation of Fibronectin (FN).

[0187] Pal-Tetra-7 was loaded into milk exosome with freeze/thaw. The fibroblast cells or keratinocytes were treated with either peptide only or loaded exosome for 72 hours and then the cells were collected and followed by total RNA isolation and qRT-PCR. The expression (fold increase) of collagen I, elastin and fibronectin in peptide only or loaded exosomes were compared with media alone and graphed.

[0188] Fibroblasts: As shown in Figure 4A-C, 1 of 3 fibroblast donors showed up-regulation of Collagen I (COL1) with treatment of loaded exosome (Exo Loaded) compared with peptide only sample. 2 of 3 did not give positive response at the tested concentration. 1 of 3 fibroblast donors showed up-regulation of Elastin (ELN). 1 of 3 fibroblast donors showed up-regulation of Fibronectin (FN).

[0189] Keratinocyte: As shown in Figure 4D-E, 2 of 3 keratinocytes donors showed up-regulation of Collagen I (COL1) with treatment of loaded exosome (Exo Loaded) compared with

peptide only sample. 1 of 3 showed moderate up-regulation. 1 of 3 Keratinocyte donors showed up-regulation of Fibronectin (FN), 1 of 3 showed moderate upregulation.

[0190] Pal-Penta-4 was loaded into milk exosome with freeze/thaw. The fibroblast cells or keratinocytes were treated with either peptide only or loaded exosome for 72 hours and then the cells were collected and followed by total RNA isolation and qRT-PCR. The expression (fold increase) of collagen I and fibronectin in peptide only or loaded exosomes were compared with media alone and graphed.

[0191] Fibroblasts: As shown in Figure 5A-C, 3 of 6 fibroblast donors showed up-regulation of Collagen I (COL1) with treatment of loaded exosome (Exo Loaded) compared with peptide only sample. 3 of 6 fibroblast donors showed up-regulation of Elastin (ELN). 3 of 6 fibroblast donors showed up-regulation of Fibronectin (FN).

[0192] Keratinocyte: As shown in Figure 5D-E, 4 of 6 keratinocytes donors showed up-regulation of Collagen I (COL1) with treatment of loaded exosome (Exo Loaded) compared with peptide only sample. 1 of 6 showed moderate up-regulation. 6 of 6 Keratinocyte donors showed up-regulation of Fibronectin (FN).

[0193] Pal-Hexa-12 was loaded into milk exosome with freeze/thaw. The fibroblast cells or keratinocytes were treated with either peptide only or loaded exosome for 72 hours and then the cells were collected and followed by total RNA isolation and qRT-PCR. The expression (fold increase) of collagen I, elastin and fibronectin in peptide only or loaded exosomes were compared with media alone and graphed.

[0194] Fibroblasts: As shown in Figure 6A-C, 4 of 6 fibroblast donors showed up-regulation of Collagen I (COL1) with treatment of loaded exosome (Exo Loaded) compared with peptide only sample. 2 of 6 showed moderate up-regulation. 2 of 6 fibroblast donors showed up-regulation of Elastin (ELN). 3 of 6 fibroblast donors showed up-regulation of Fibronectin (FN).

[0195] Keratinocyte: As shown in Figure 6D-E, 3 of 6 keratinocytes donors showed up-regulation of Collagen I (COL1) with treatment of loaded exosome (Exo Loaded) compared with peptide only sample. 2 of 6 showed moderate up-regulation. 1 of 6 did not show positive

response. 5 of 6 Keratinocyte donors showed up-regulation of Fibronectin (FN). 1 of 6 showed moderate upregulation.

[0196] Non responder Experiments

[0197] Multiple donor fibroblast and donor keratinocyte that did not respond to skin peptide treatment were identified. Those donors, aHDF450, aHDF361, aHK973, aHK653, aHK318, were stimulated when treated with Pal-Tri1 and –Tri38 loaded milk exosomes. See Figure 7A and 7B. In addition, a dose-dependent response was observed in fibroblast donor aHDF450 when treated with various concentrations of Pal-Penta4 loaded milk exosomes.

[0198] Stability

[0199] Peptide Stability

[0200] Two different lots of milk exosomes were used for loading and were diluted to 1E+13 particles/mL in Dulbecco's phosphate-buffered saline (DPBS). Pal-Tetra7-OH was diluted from 2mg/mL stock to 0.25 mg/mL using DPBS. The milk exosome only or mixed sample was treated by one of four loading methods (co-incubation, extrusion, freeze-thaw, and sonication). For each loaded sample, 100 µL was loaded onto an iZon qEV single SEC column. Using DPBS as eluent, exosome fractions (200 µL fractions, Fr. 6-8) were collected. For each sample, one aliquot (108 µL) of exosome fractions was lysed in 1% SDS, RT overnight (>16 hrs) at each time point. All samples were analyzed by HPLC and skin peptide recovery was measured as a % normalized to day 0 result.

[0201] Pal-Tri38 loaded milk exosomes, Pal-Tri1 loaded milk exosomes, Pal-Penta4 loaded milk exosomes, Pal-Hexa12 loaded milk exosomes, Pal-Tetra7 and Pal-Tri38 loaded exosomes were tested for stability at -20°C or 4°C for various time points as described.

[0202] Pal-Tetra7-OH was detected in exosome fractions of loaded samples after storage at -20 °C or 4 °C at all various time points tested. For various exosome lots, loaded Pal-Tetra7-OH were stable (>80%) at -20 °C or 4 °C at the 1 month and 3 month time points.

[0203] Pal-Tri38-NH2 was detected in exosome fractions of loaded samples after storage at -20°C or 4°C at various time points. Except for Pal-Tri38 loaded with in milk exosomes using

sonication, loaded Pal-Tri38-NH2 was stable (>80%) at -20°C for at least 1 month. For milk exosome lot LSAK7 and LSAK8, loaded Pal-Tri38-NH2 was stable (>80%) at 4°C for at least 1 month or 3 months.

[0204] Pal-Tri1-NH2 was detected in exosome fractions of loaded samples after storage at -20°C or 4°C for various time points. For milk exosome lot LSAK14 and LSAK18, loaded Pal-Tri1-NH2 was stable (>80%) at -20 °C or 4 °C for at least 1 month.

[0205] Pal-Penta4-NH2 was detected in exosome fractions of loaded samples after storage at -20 °C or 4 °C for various time points. For milk exosome lot LSAK14 and LSAK18, loaded Pal-Penta4-NH2 was stable (>80%) at -20 °C for at least 1 month. Except for Pal-Penta4 loaded with milk exosomes using co-incubation and freeze-thaw, loaded Pal-Penta4-NH2 was stable (>80%) at 4°C for at least 1 month.

[0206] Pal-Hexa12-NH2 was detected in exosome fractions of loaded samples after storage at -20 °C or 4 °C for various time points. For exosome lot LSAK14 and LSAK18, loaded Pal-Hexa12-NH2 was stable (>80%) at -20 °C or 4°C for at least 1 month.

[0207] The combination of Pal-Tetra7-OH and Pal-Tri38-NH2 was detected in exosome fractions of loaded samples after storage at -20°C or 4°C for various time points. For exosome lot LSAK14 and LSAK18, loaded Pal-Tetra7-OH and Pal-Tri38-NH2 was stable (>80%) at -20°C and at 4°C for at least 1 month. For exosome lot LSAK14, time-dependent degradations of loaded Pal-Tetra7-OH were observed at 4 °C for all loading methods. For exosome lot LSAK18, loaded Pal-Tetra7-OH was stable (>80%) at 4 °C for at least 1 month.

[0208] Exosome Stability

[0209] Milk exosomes were isolated as described before. Various skin peptides (0.25 mg/ml in 0.5 mL) were loaded into different lots of exosomes via sonication or freeze thaw. Controls (exosomes only) in water or PBS were used. 2 lots of exosomes were tested for each storage condition that included -20°C, 4°C, room temperature, 37°C and 40°C and at time points of D0, D7, D14, D30, D90 and D180. Loaded exosomes were analyzed by: 1) Nanosight ; 2) HPLC for loading stability, 3) Western blot (presence of proteins).

[0210] Pal-Tri38 was loaded onto several lots of milk exosomes (LSAK39, LKSAK42) and the stability of the loaded exosomes in comparison with untreated exosomes was examined. All exosomes (loaded and unloaded) at similar concentrations was detectable after 1 month at different temperatures (4°C, RT, 37°C and 40°C). Pal-Tri38 loaded in two different lots of exosomes was also found to be stable after 1 month at different temperatures (4°C, RT, 37°C and 40°C). A marker for exosome, CD63, was still detectable via Western blot for at least 1 month at different temperatures (4°C, RT, 37°C and 40°C), suggesting that the loaded milk exosomes were stable for at least a month.

[0211] Exosome Uptake

[0212] The uptake of exosomes in 2D cell culture was analyzed by flow cytometry. Briefly, dye-labeled exosomes were added to cells. Cells were then lifted and ran on flow cytometer to detect presence of dye. % uptake = % cells containing dye labeled exosomes.

[0213] 1.1E+14 milk exosomes were loaded with Pal-Tri38 using one of each of the 4 loading methods (co-incubation, freeze thaw, extrusion or sonication). The loaded exosomes were healed overnight, and then labeled with DiO (3,3'-Dioctadecyloxacarbocyanine Perchlorate) per manufacturer's protocol. Exosomes were then concentrated, added to exosome spin column, and counted on the Nanosight. Two 1:100 serial dilutions were made to make the two lower doses.

[0214] Labeled exosomes were co-incubated with aHEK 973 or aHDF576 cells in duplicates in 300uLfor 2 hours at 37°C with1E+13, 1E+11, 1E+9 exosomes/mL in HK media. Cells were then dissociated with TrypLE™ (Thermo Fisher) for 5 mins , washed and run on the iQue. Analysis was completed with FlowJo. Gating was based on labeled PBS+aHEK for controls.

[0215] Pal-Tri-38 loaded milk exosomes uptake in keratinocytes: At E+9 exosome /ml, the uptake efficiency for keratinocyte ranged from ~2-5%. At E+11 exosome/ ml, the uptake efficiency for keratinocyte ranged from ~32.5-90%. At E+13 exosome/ml, the uptake efficiency for keratinocyte ranged from ~99.5-100%. Different loading methods demonstrated similar uptake efficiencies although at E+11, the sonication samples provided lower uptake (~58.5%) compared with the other methods (~75.5-90%). The uptake efficiency however was still higher than exosome only sample (~32.5%) at E+11. See Figure 8A.

[0216] Pal-Tri-38 loaded milk exosomes uptake in fibroblasts: At E+9 exosome /ml, the uptake efficiency of loaded exosomes in fibroblast ranged from ~2-3%. At E+11 exosome/ ml, the uptake efficiency for loaded exosomes in fibroblast ranged from ~42.5-95%. At E+13 exosome/ml, the uptake efficiency for loaded exosomes in fibroblast was at ~100%. Different loading methods demonstrated similar uptake efficiencies although at E+11, sonication samples provided lower uptake (~42.5%) compared with the other methods (~85-95%). See Figure 8B.

[0217] Pal-Tetra7 loaded milk exosomes uptake in keratinocytes: At E+9 exosome /ml, the uptake efficiency for loaded exosomes in keratinocytes ranged from ~2-5.5%. At E+11 exosome/ ml, the uptake efficiency for loaded exosomes in keratinocytes ranged from ~32.5-88%. At E+13 exosome/ml, the uptake efficiency for loaded exosomes in keratinocytes was at ~100%. Different loading methods demonstrated similar uptake efficiencies although at E+11, coincubation samples provided lower uptake (~49%) compared with the other methods (~78.5-88%). See Figure 8C.

[0218] Pal-Tetra7 loaded milk exosomes uptake in fibroblasts: At E+9 exosome /ml, the uptake efficiency for loaded exosomes in fibroblast ranged from ~2-2.5%. At E+11 exosome/ ml, the uptake efficiency for loaded exosomes in fibroblast ranged from ~49.5-95%. At E+13 exosome/ml, the uptake efficiency for loaded exosomes in fibroblast was at ~100%.Different loading methods demonstrated similar uptake efficiencies although at E+11, sonication samples provided lower uptake (~49.5%) compared with the other methods (~92.5-95%). See Figure 8D.

[0219] Skin Models

[0220] 3D reconstituted skin model – milk exosome penetrance

[0221] 3D reconstituted neonatal and adult skin with distinct dermis, epidermis and stratum corneum layers were used. Fluorescently labelled-milk exosomes were applied topically. Penetration of the milk exosomes into the dermis layer was observed (data not shown). Exosome penetrance into the dermis was observed in 34 explants in 5 studies. Increased penetrance was also observed with additional dosing. Similar results were observed for both adult and neonatal explants.

[0222] 3D reconstituted skin model – skin peptide loaded milk exosome

[0223] Milk exosomes were isolated as described before. Fluorescently labeled skincare peptides were loaded into different lots of exosomes via sonication or freeze thaw. Controls

(exosomes only) in water or PBS were used. Figure 9A shows loaded milk exosomes efficiently deliver labeled skincare peptides into all 3 skin layers, including the dermis. Milk exosome delivery of peptides to the dermis was observed in 24 skin explants in 3 studies. Figure 9A further shows that labeled peptide alone remained in the stratum corneum when applied to the 3D skin.

[0224] Adult skin explants- milk exosome penetrance

[0225] Fluorescently labelled-milk exosomes were applied topically. Penetration of the milk exosomes into the dermis layer of the adult skin explants was observed (data not shown). Exosome delivery of peptides to the dermis observed in 14 skin explants in 4 studies (data not shown)

[0226] Adult skin explants-– skin peptide loaded milk exosome

[0227] Fluorescently labelled-milk exosomes were applied topically on adult skin explants. Figure 9B shows loaded milk exosomes penetrate and deliver peptides into all three skin layers, including the dermis of explants. Milk exosome delivery of peptides to the dermis was observed in 14 skin explants in 3 studies. Figure 9B further shows that labeled peptide alone remained in the stratum corneum when applied to the adult skin explants.

[0228] Emulsifiers

[0229] To investigate total peptide stability in different emulsifiers, two lots of exosomes were loaded with Pal-Tri1. Loaded exosomes were mixed with different emulsifiers (1% Tween-80, 2% Lipowax D, 2% Arlacel 165, or 2% sodium stearate) plus 1% Mikrokill as preservative. Aliquots of different samples were stored at room temperature and 40°C for 3 months. At each time point (0, 1, 2, 3-month), aliquots of all samples were analyzed by HPLC and the total peptide concentrations were normalized to respective results at day 0 (as 100%) for each group. For 2% sodium stearate, the samples were too viscous to analyze on HPLC, therefore no data for this group were collected.

[0230] To test whether loaded peptide remained in exosomes in different emulsifiers, two lots of exosomes were loaded with Pal-Tri1. Loaded exosomes were mixed with different emulsifiers (1% Tween-80, 2% Lipowax D, or 2% Arlacel 165). Aliquots of different samples were stored at 4°C, room temperature, and 40°C for 3 or 6 months. At each time point (day 0, 1, 2, 3, or 6-month), loaded exosomes were purified from 100 μL of each sample before analysis by HPLC.

The loaded peptide concentrations in exosome fractions were normalized to respective results at day 0 (as 100%) for each group.

[0231] For penetrance test, exosomes were loaded with Pal-Tri1-TMR. After purification, loaded exosomes were mixed with different emulsifiers (1% Tween-80, 2% Lipowax D, 2% Arlacel 165, or 2% sodium stearate) plus 1% Mikrokill as preservative. Different samples were stored at 40°C for 3 months. At each time point (day 0, 1, 2, 3-month), a penetrance assay was performed using in-house 3D skin equivalents. Briefly, 50 µL of each sample (Pal-Tri1-TMR at 25 µM) was dosed daily for 48 hours, then skin samples were frozen in OCT media and cryosectioned. Images were taken and analyzed with Nikon software. This assay was designed to examine the functionality of loaded exosomes (penetrance through skin) after 3-month storage at 40°C.

[0232] Results (data not shown)

[0233] Lipowax D was observed to have the best compatibility with loaded exosomes among all emulsifiers tested.

[0234] A peptide was considered stable at the storage condition when the remaining peptide in sample was ≥ 60%. Total peptide was stable for 3 months at 40°C in most conditions tested for Tween-80, Lipowax D, and Arlacel 165. Sodium stearate was tested but too viscous to analyze by HPLC.

[0235] Loaded peptide in exosome remained stable after 3-month storage at 40°C with Lipowax D. However, significant leakage from exosomes was observed with Tween-80 and Arlacel 165

[0236] All 4 emulsifiers conditions showed some amount of penetrance in both donors tested at 3-month time point.

[0237] Unless defined otherwise, all technical and scientific terms and any acronyms used herein have the same meanings as commonly understood by one of ordinary skill in the art in the field of this invention.

[0238] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the present disclosure. It should be understood that various alternatives to the embodiments described herein, or combinations of one or more of these embodiments or aspects described therein may be employed in practicing the present disclosure. It is intended that the following claims define the scope of the present disclosure and

that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

1. An exosome comprising at least one skin care peptide.

2. A milk-derived exosome comprising at least one skin care peptide.

3. The exosome of claim 2, wherein milk is bovine milk.

4. The exosome of claims 1 or 2, wherein the skin care peptide is Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-EEMQRRAD); Copper Tripeptide-1 (Copper-GHK); Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2, Tetrapeptide-21 or any combination thereof.

5. The exosome of claim 1 or 2, wherein at least one of the skin care peptide is Palmitoyl Pentapeptide-4.

6. The exosome of claim 1 or 2, wherein at least one of the skin care peptide is Palmitoyl Tetrapeptide-7.

7. The exosome of claim 1 or 2, wherein at least one of the skin care peptide is Palmitoyl Tripeptide-38.

8. The exosome of claim 1 or 2, wherein at least one of the skin care peptide is Palmitoyl Tripeptide-1.

9. The exosome of claim 1 or 2, wherein at least one of the skin care peptide is Palmitoyl Hexapeptide-12.

10. A composition comprising the exosome of any one of claims 1-9 and a physiologically acceptable medium.

11. An anti-wrinkle cosmetic composition comprising an effective amount of exosomes, wherein the exosomes comprise at least one skin care peptide.

12. An anti-aging cosmetic composition comprising an effective amount of exosomes, wherein the exosomes further comprise at least one skin care peptide.

13. The composition of claims 11 or 12 wherein the composition is a topical composition.

14. The composition of claims 11 or 12, wherein the skin care peptide is Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-EEMQRRAD); Copper Tripeptide-1 (Copper-GHK); Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2, Tetrapeptide-21 or any combination thereof.

15. The composition of claims 11 or 12, wherein at least one of the skin care peptide is Palmitoyl Pentapeptide-4.

16. The composition of claims 11 or 12, wherein at least one of the skin care peptide is Palmitoyl Tetrapeptide-7.

17. The composition of claims 11 or 12, wherein at least one of the skin care peptide is Palmitoyl Tripeptide-38.

18. The composition of claims 11 or 12, wherein at least one of the skin care peptide is Palmitoyl Tripeptide-1.

19. The composition of claims 11 or 12, wherein at least one of the skin care peptide is Palmitoyl Hexapeptide-12.

20. A method of obtaining milk-derived exosome loaded with skin care peptides comprising:
    a. isolating exosomes from milk; and
    b. introducing at least one skin care peptide directly into the isolated exosomes.

21. The method of claim 20, wherein introducing at least one skin care peptide into the isolated exosomes comprises freezing and thawing the isolated exosomes and at least one skin care peptide.

22. The method of claim 20, wherein introducing at least one skin care peptide into the isolated exosomes comprises coincubating the isolated exosomes and at least one skin care peptide.

23. The method of claim 20, wherein introducing at least one skin care peptide into the isolated exosomes comprises sonicating the isolated exosomes and at least one skin care peptide.

24. The method of claim 20, wherein introducing at least one skin care peptide into the isolated exosomes comprises extrusion of the isolated exosomes and at least one skin care peptide.

25. The method of claim 20, wherein the milk is bovine milk.

26. The method of claim 20, wherein the skin care peptide is Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-EEMQRRAD); Copper Tripeptide-1 (Copper-GHK); Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2, Tetrapeptide-21 or any combination thereof.

27. The method of claim 20, wherein at least one of the skin care peptide is Palmitoyl Pentapeptide-4.

28. The method of claim 20, wherein at least one of the skin care peptide is Palmitoyl Tetrapeptide-7.

29. The method of claim 20, wherein at least one of the skin care peptide is Palmitoyl Tripeptide-38.

30. The method of claim 20, wherein at least one of the skin care peptide is Palmitoyl Tripeptide-1.

31. The method of claim 20, wherein at least one of the skin care peptide is Palmitoyl Hexapeptide-12.

32. A method of treating skin comprising topically applying to skin of a subject in need thereof, a composition comprising an effective amount of exosomes, wherein the exosomes comprise at least one skin care peptide.

33. The method of claim 32, wherein the composition is applied to facial skin.

34. The method of claim 32, wherein the composition is applied to a fine line or wrinkle.

35. The method of claim 32, wherein the composition is a cream or a lotion.

36. The method of claim 32, wherein the composition moisturizes skin.

37. The method of claim 32, wherein the composition increases the expression of collagen 1.

38. The method of claim 32, wherein the composition increases the expression of fibronectin.

39. The method of claim 32, wherein the composition is applied at least once a day.

40. The method of claim 32, wherein the composition is applied before sleep.

41. The method of claim 32, wherein the composition is non-comedogenic.

42. The method of claim 32, wherein the skin is erythemic, sensitive, or inflamed skin,

43. The method of claim 32, wherein the skin care peptide is Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-EEMQRRAD); Copper Tripeptide-1 (Copper-GHK); Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2, Tetrapeptide-21 or any combination thereof.

44. The method of claim 32, wherein at least one of the skin care peptide is Palmitoyl Pentapeptide-4.

45. The method of claim 32, wherein at least one of the skin care peptide is Palmitoyl Tetrapeptide-7.

46. The method of claim 32, wherein at least one of the skin care peptide is Palmitoyl Tripeptide-38.

47. The method of claim 32, wherein at least one of the skin care peptide is Palmitoyl Tripeptide-1.

48. The method of claim 32, wherein at least one of the skin care peptide is Palmitoyl Hexapeptide-12.

49. The method of claim 32, wherein a subject in need thereof is a non-responder.

50. A method of delivering at least one skin care peptide to a dermis layer of skin comprising applying a composition comprising an effective amount of exosomes to skin, wherein the exosomes comprise at least one skin care peptide.

51. The method of claim 50 wherein the composition is applied to facial skin.

52. The method of claim 50, wherein the composition is applied to a fine line or wrinkle.

53. The method of claim 50, wherein the composition is a cream or a lotion.

54. The method of claim 50, wherein the composition moisturizes skin.

55. The method of claim 50, wherein the composition increases the expression of collagen 1.

56. The method of claim 50, wherein the composition increases the expression of fibronectin.

57. The method of claim 50, wherein the composition is applied at least once a day.

58. The method of claim 50, wherein the composition is applied before sleep.

59. The method of claim 50, wherein the composition is non-comedogenic.

60. The method of claim 50, wherein the skin is erythemic, sensitive, or inflamed skin.

61. The method of claim 50, wherein the skin care peptide is Palmitoyl Pentapeptide-4 (Palmitoyl-KTTKS); Palmitoyl Tetrapeptide-7 (Palmitoyl GQPR); Palmitoyl Tripeptide-38; Palmitoyl Tripeptide-1 (Palmitoyl-GHK); Palmitoyl Hexapeptide-12 (Palmitoyl-VGVAPG); Acetyl Hexapeptide-8 (Acetyl- EEMQRR-amide); SNAP-8 Acetyl Octapeptide-3 (Acetyl-

EEMQRRAD); Copper Tripeptide-1 (Copper-GHK); Acetyl Hexapeptide-20; Palmitoyl Tripeptide-28; Hexapeptide-19; Trifluoroacetyl Tripeptide-2, Tetrapeptide-21 or any combination thereof.

62. The method of claim 50, wherein at least one of the skin care peptide is Palmitoyl Pentapeptide-4.

63. The method of claim 50, wherein at least one of the skin care peptide is Palmitoyl Tetrapeptide-7.

64. The method of claim 50, wherein at least one of the skin care peptide is Palmitoyl Tripeptide-38.

65. The method of claim 50, wherein at least one of the skin care peptide is Palmitoyl Tripeptide-1.

66. The method of claim 50, wherein at least one of the skin care peptide is Palmitoyl Hexapeptide-12.

67. The method of claim 50, wherein a subject in need thereof is a non-responder.

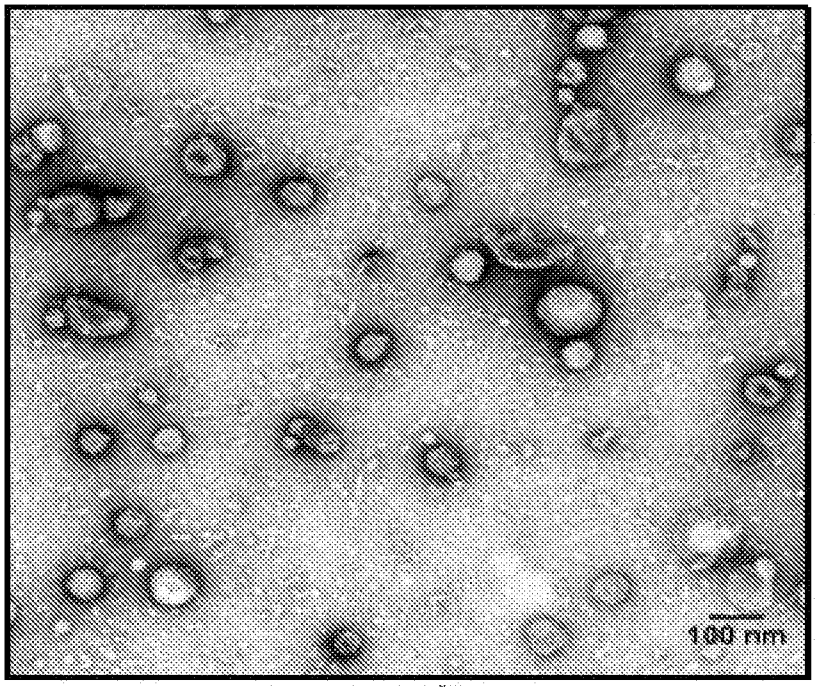
68. The composition of any one of claims 11-19, further comprising an emulsifier.

69. The composition of claim 68, wherein the emulsifier is Lipowax D.

70. The composition of any one of claims 11-19 and claims 68-69, further comprising a preservative.

71. The composition of claim 70, wherein the preservative is Mikrokill.

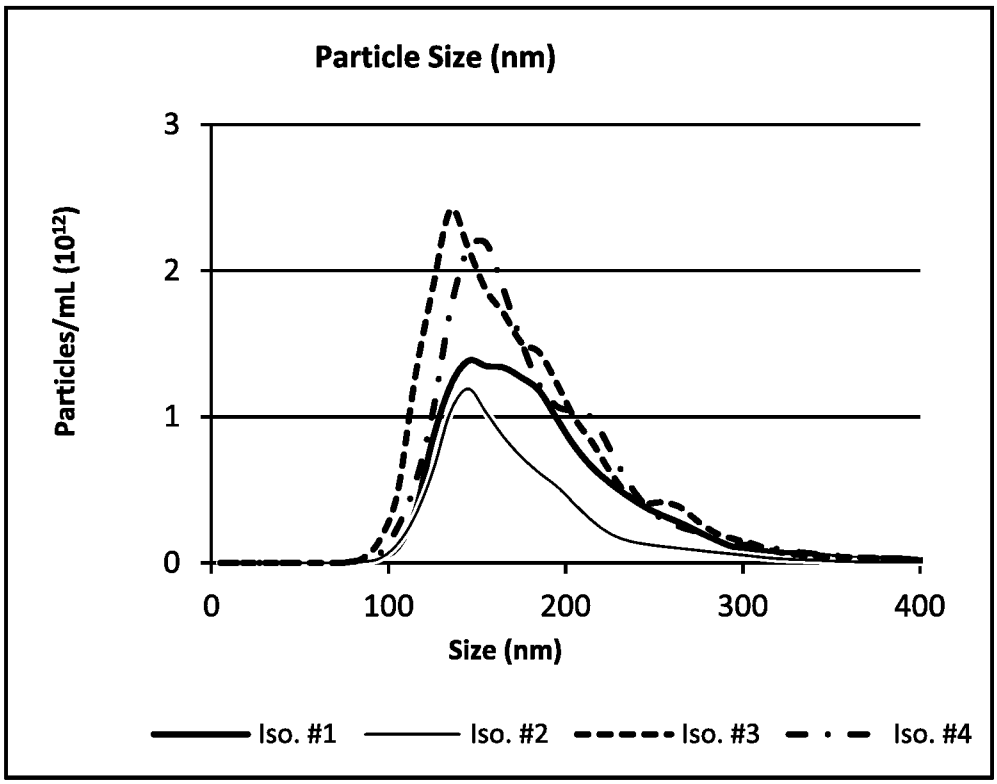
Intact Exosomes
with Expected Morphology



TEM Analysis

FIG. 1A

Proper Size Distribution of Exosomes



Nanosight Analysis

FIG. 1B

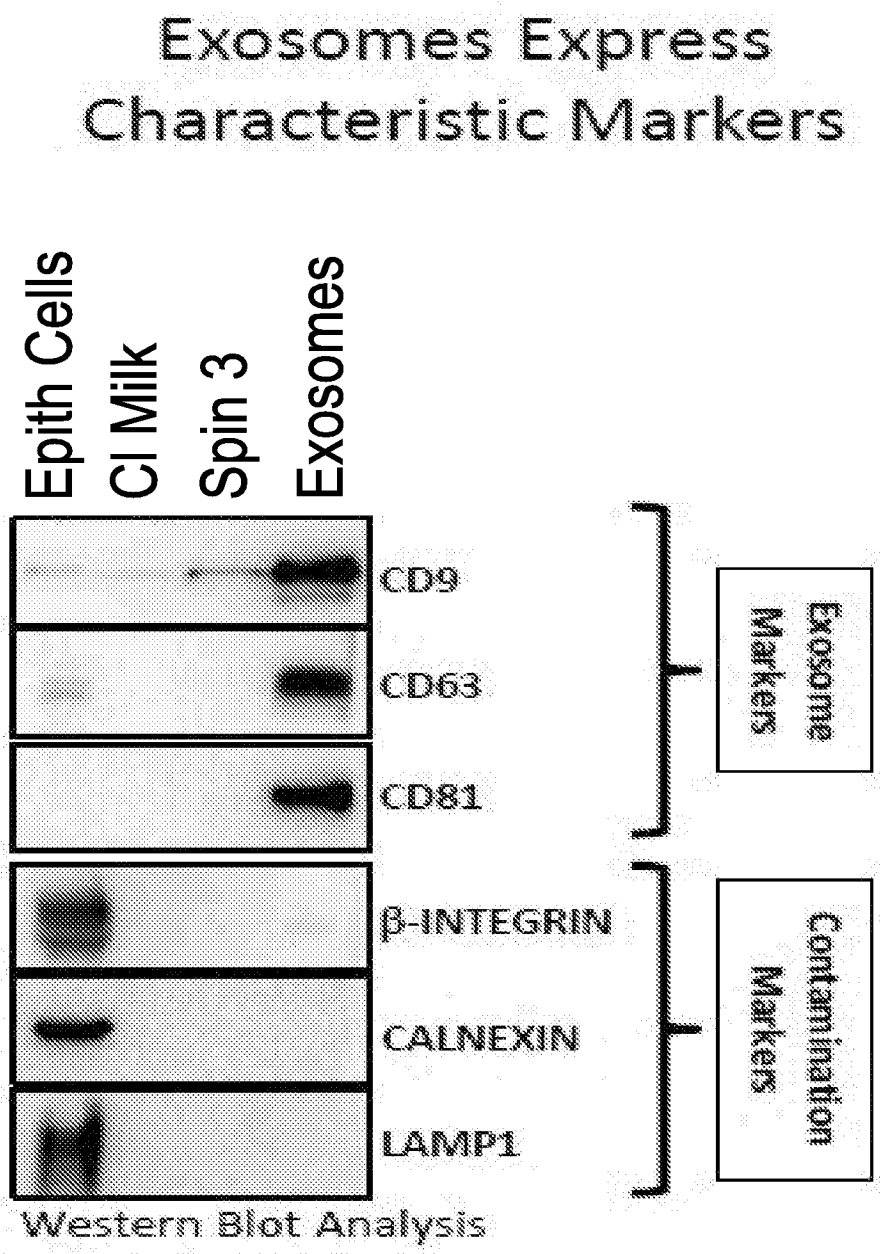
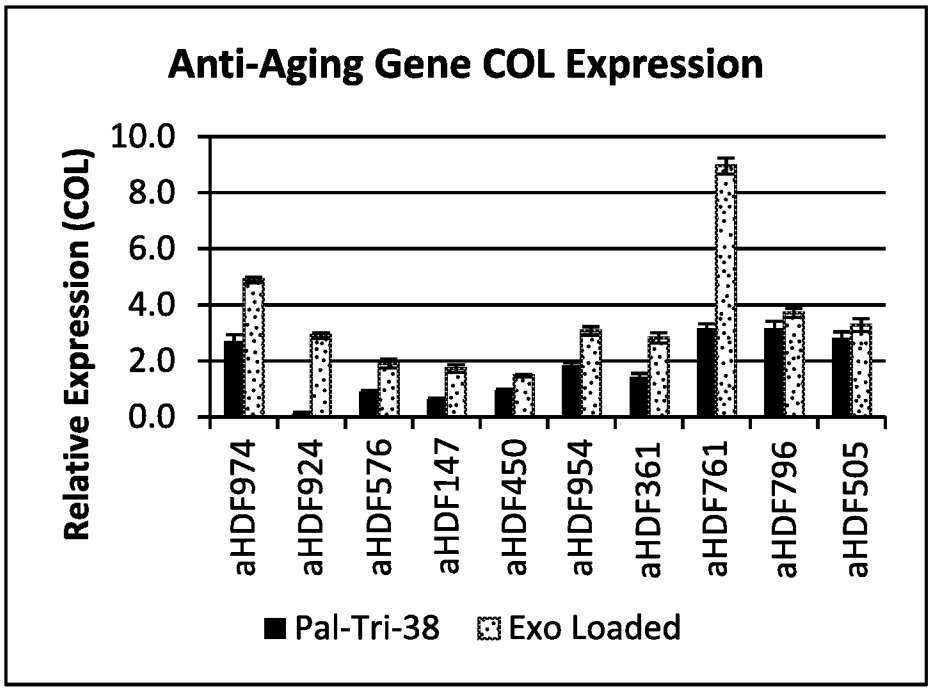
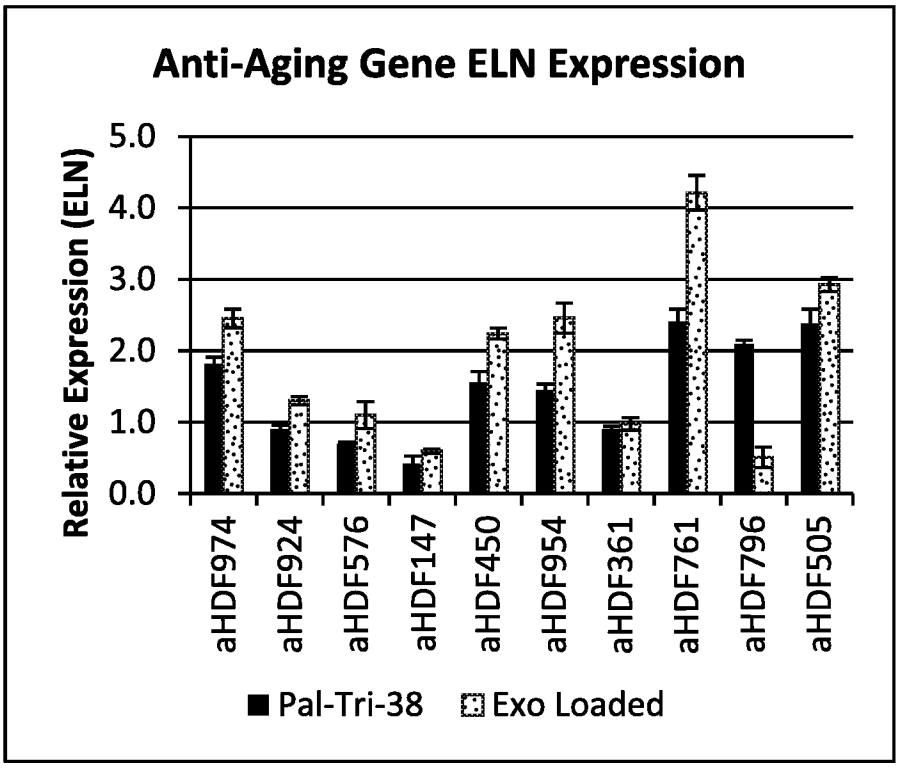

Exosomes Express
Characteristic Markers
Epith Cells
Cl Milk
Spin 3
Exosomes
CD9
CD63
CD81
β-INTEGRIN
CALNEXIN
LAMP1
Exosome Markers
Contamination Markers
Western Blot Analysis

FIG. 1C



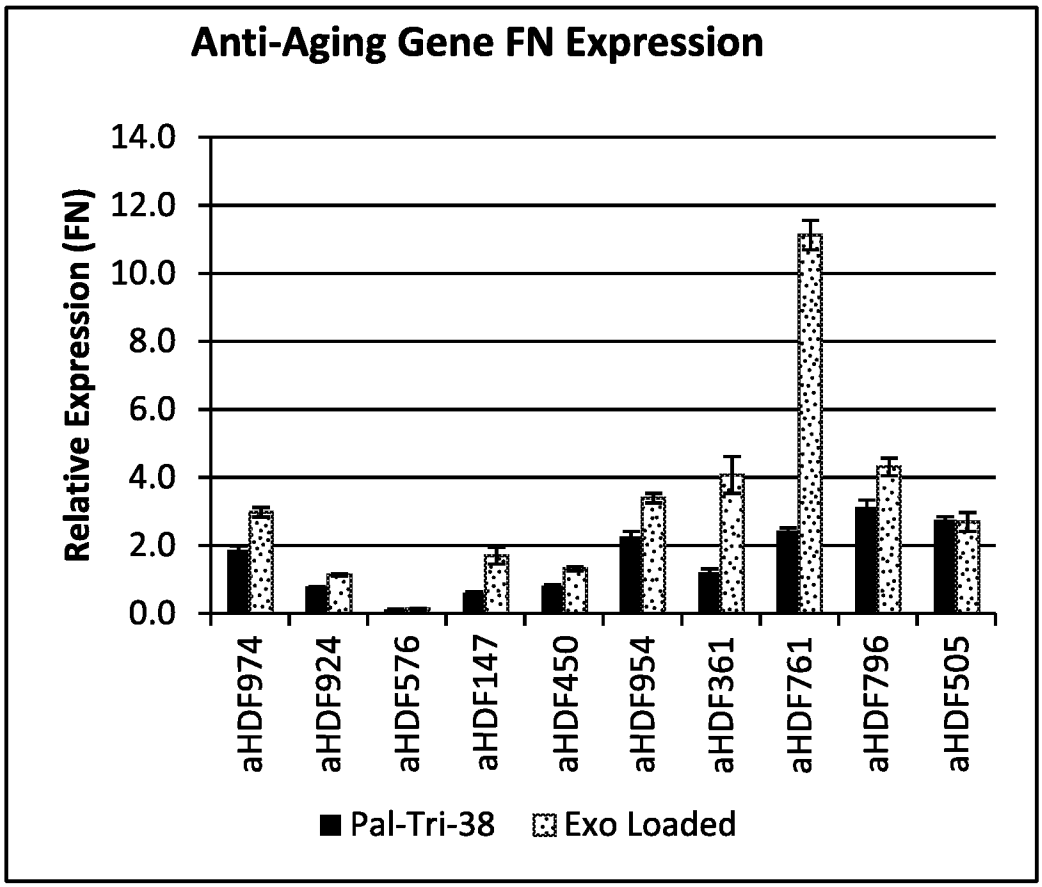
qPCR Analysis (media alone rel exp = 1)

FIG. 2A



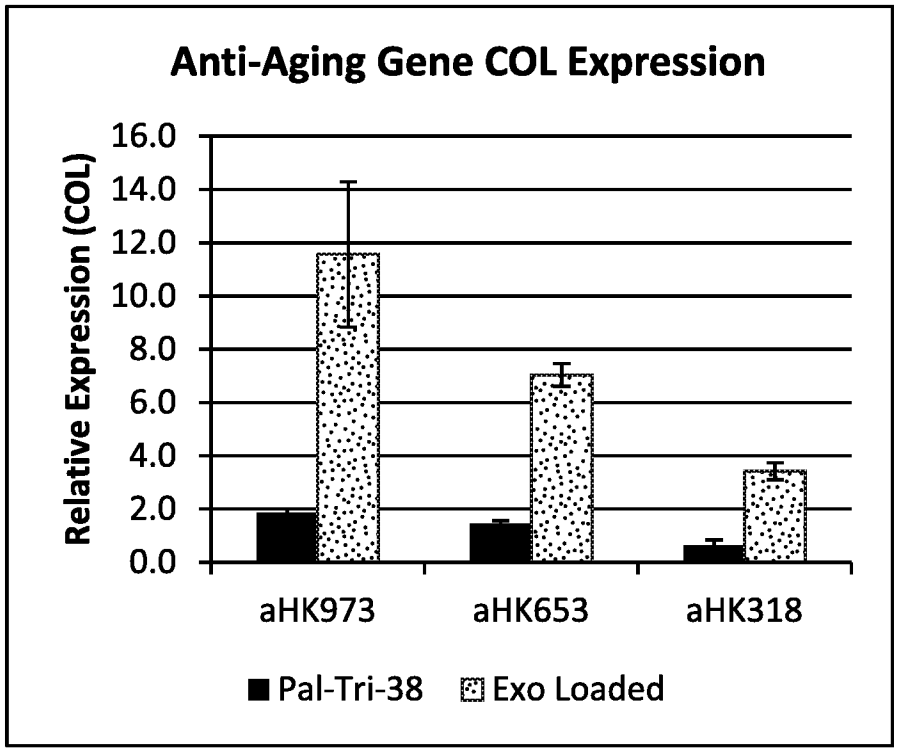
qPCR Analysis (media alone rel exp = 1)

FIG. 2B



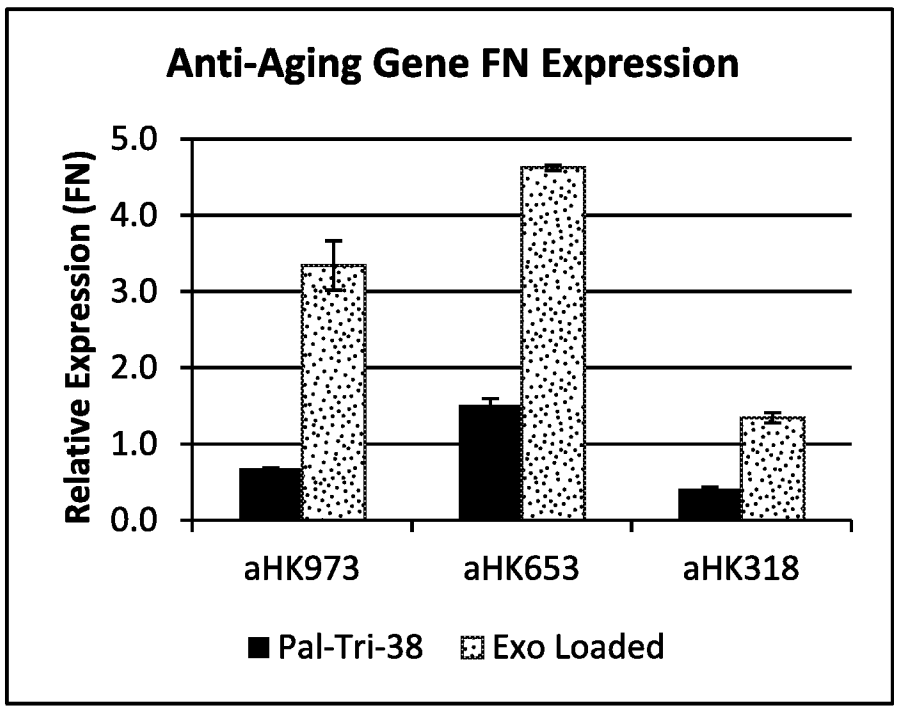
qPCR Analysis (media alone rel exp = 1)

FIG. 2C



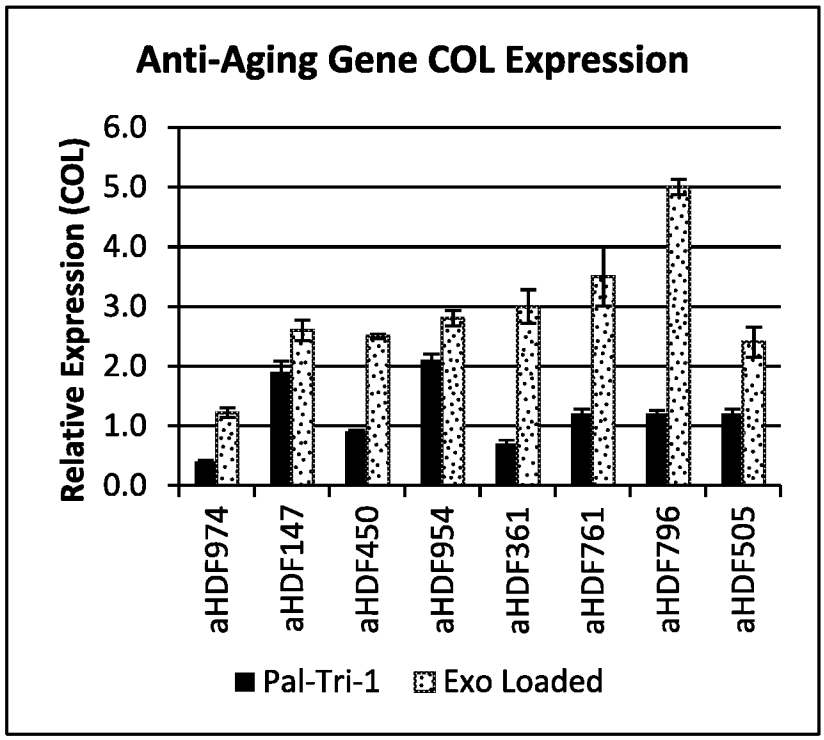
qPCR Analysis (media alone rel exp = 1)

FIG. 2D



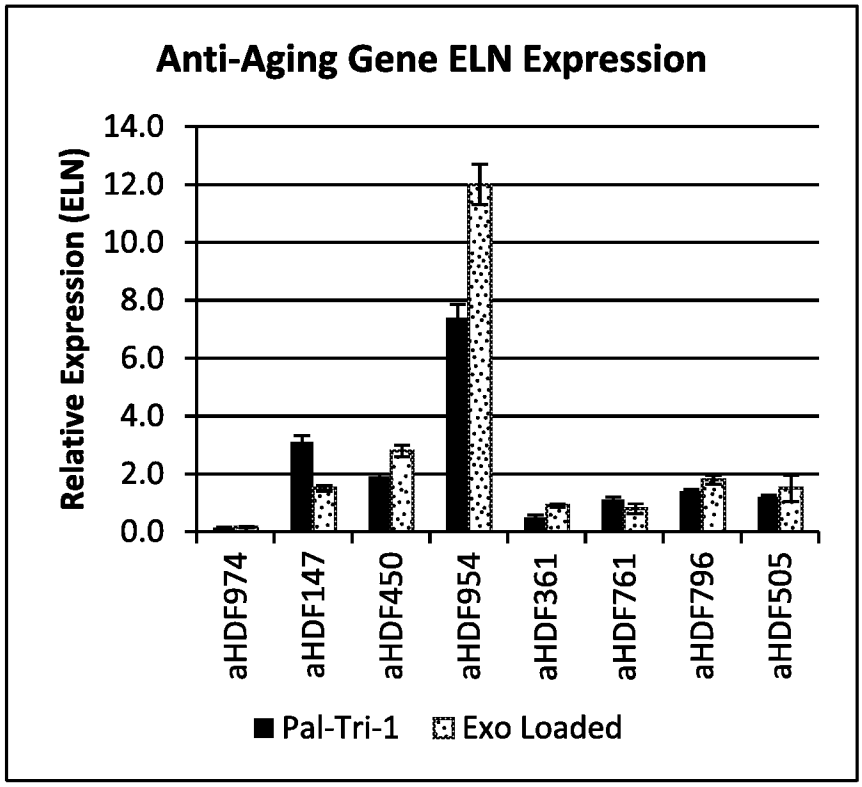
qPCR Analysis (media alone rel exp = 1)

FIG. 2E



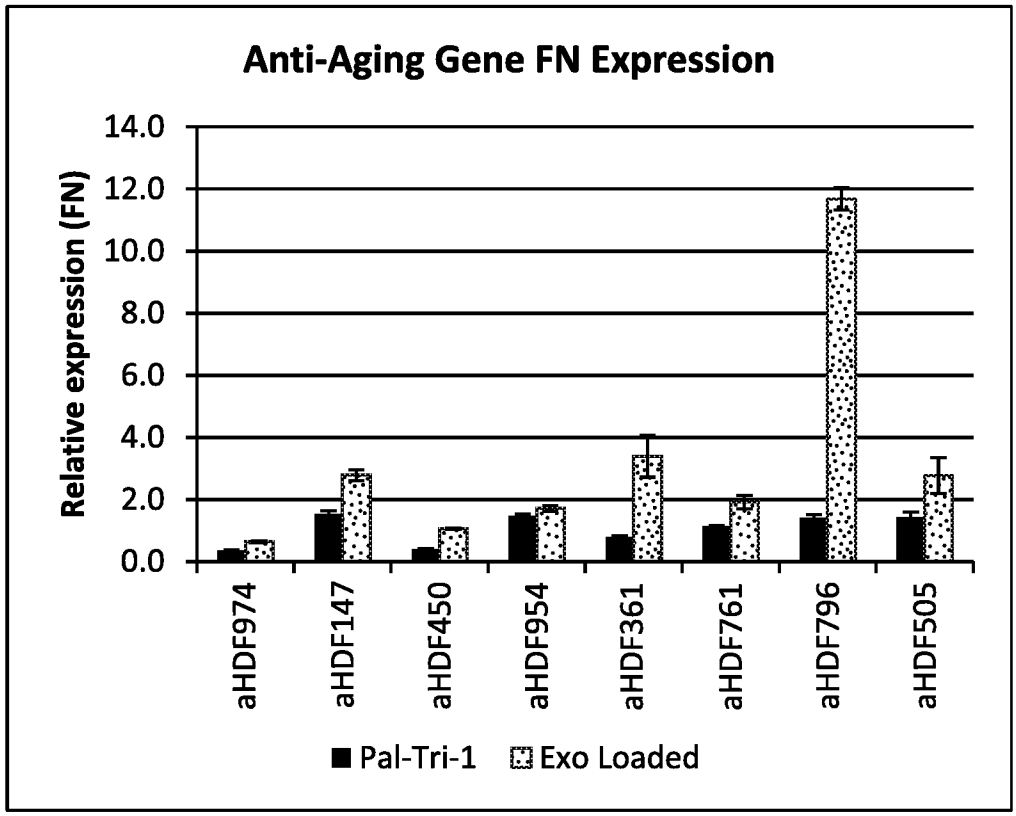
qPCR Analysis (media alone rel exp = 1)

FIG. 3A



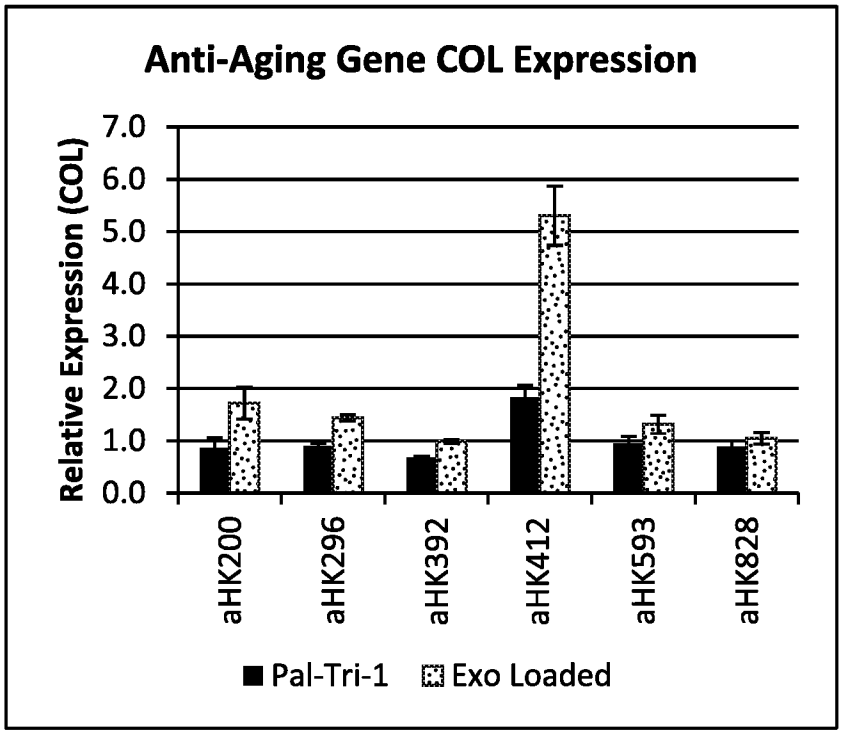
qPCR Analysis (media alone rel exp = 1)

FIG. 3B



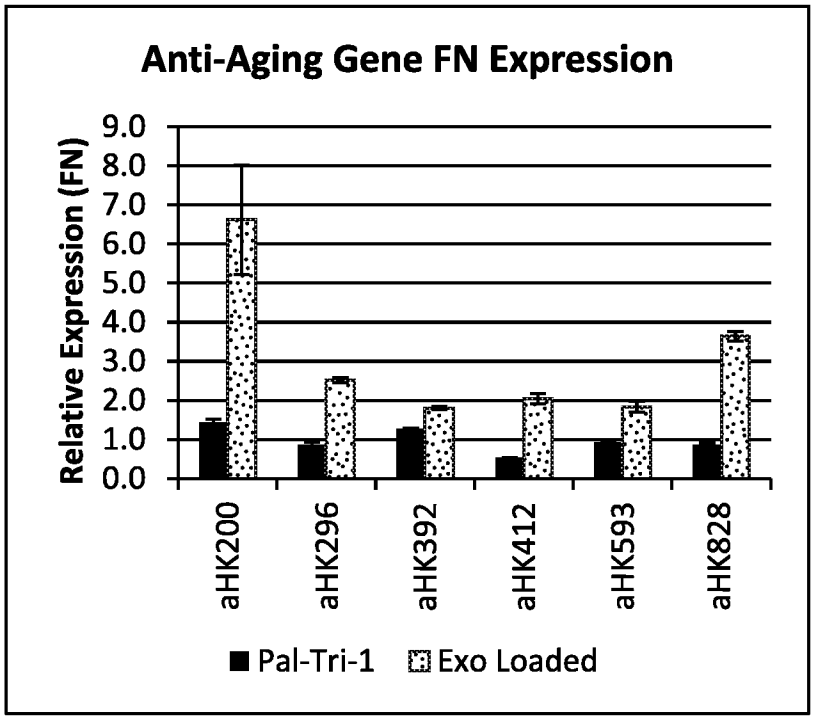
qPCR Analysis (media alone rel exp = 1)

FIG. 3C



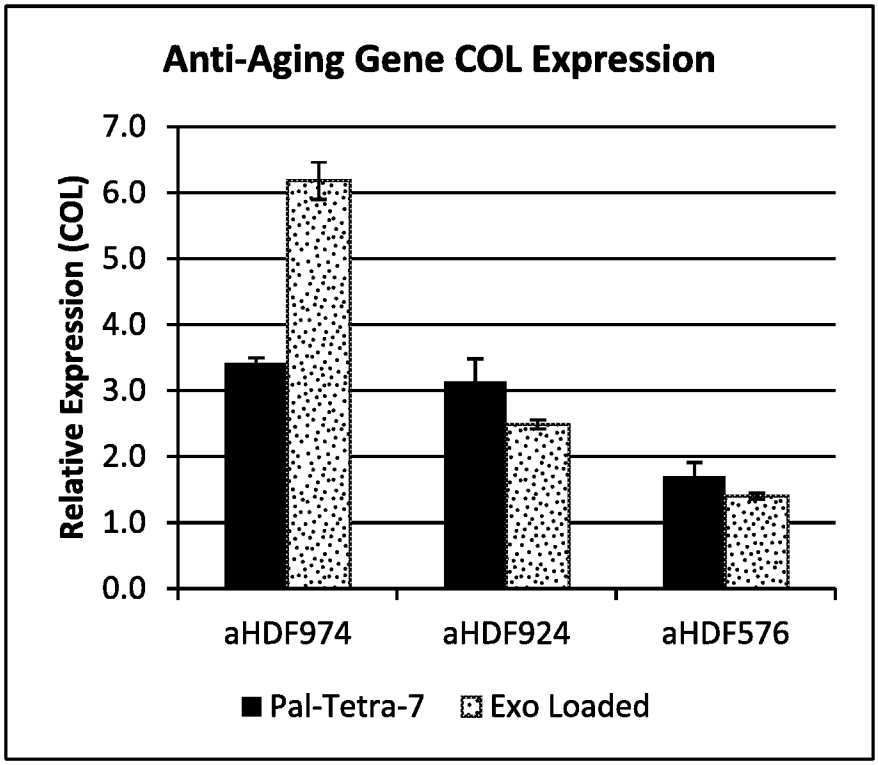
qPCR Analysis (media alone rel exp = 1)

FIG. 3D



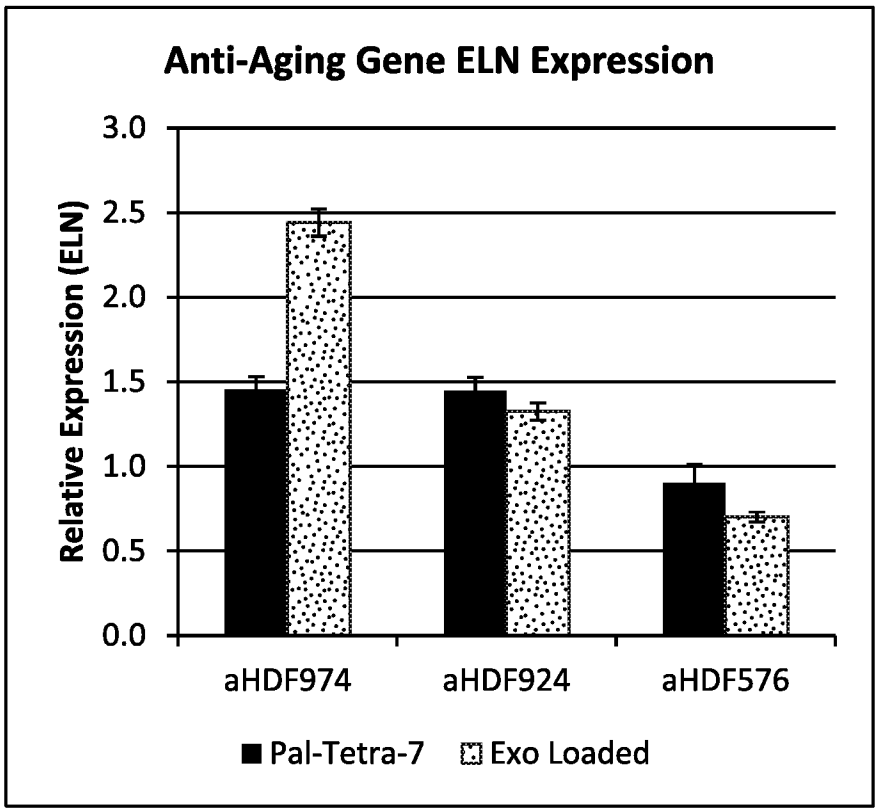
qPCR Analysis (media alone rel exp = 1)

FIG. 3E



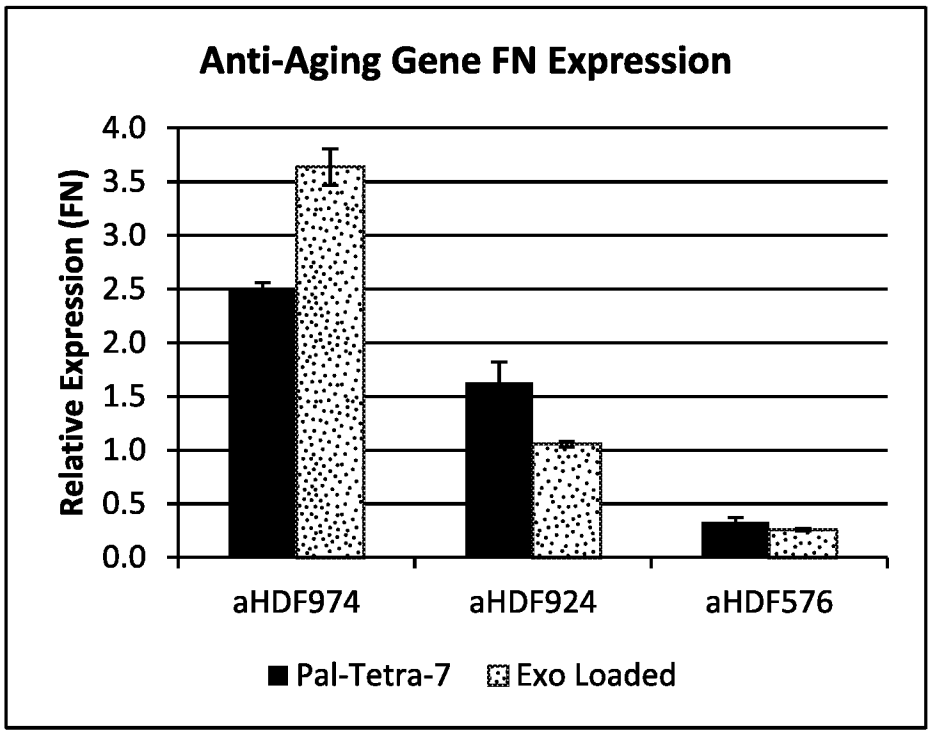
qPCR Analysis (media alone rel exp = 1)

FIG. 4A



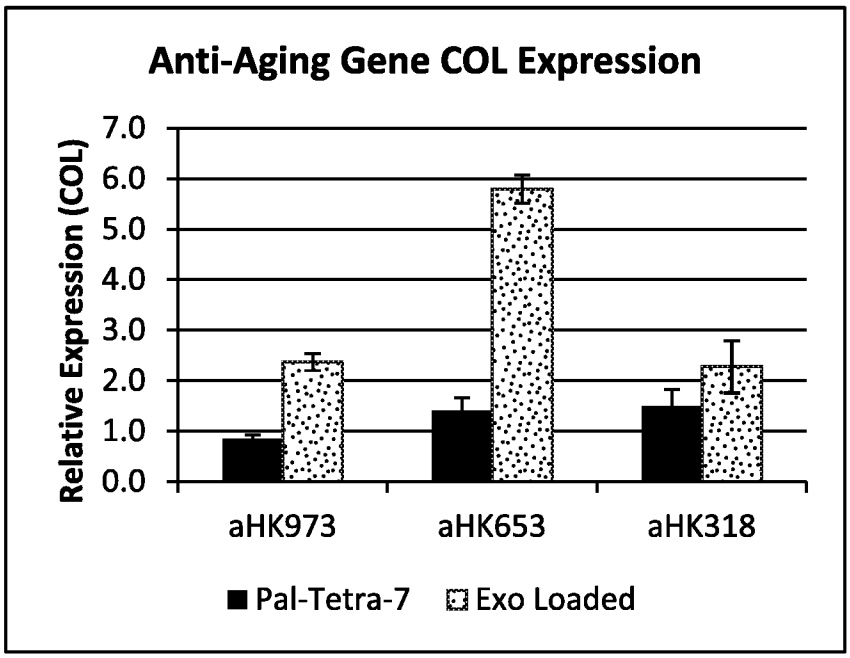
qPCR Analysis (media alone rel exp = 1)

FIG. 4B



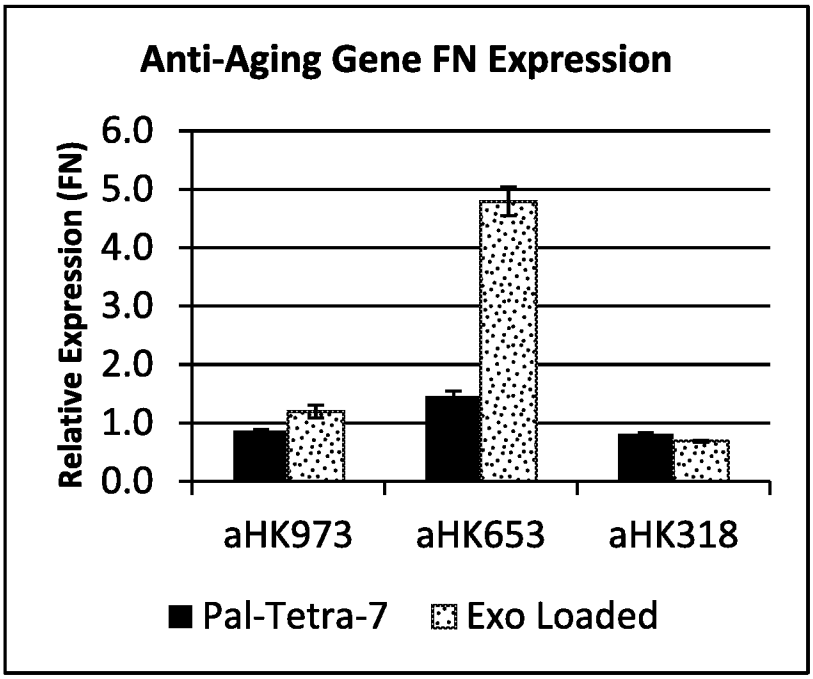
qPCR Analysis (media alone rel exp = 1)

FIG. 4C



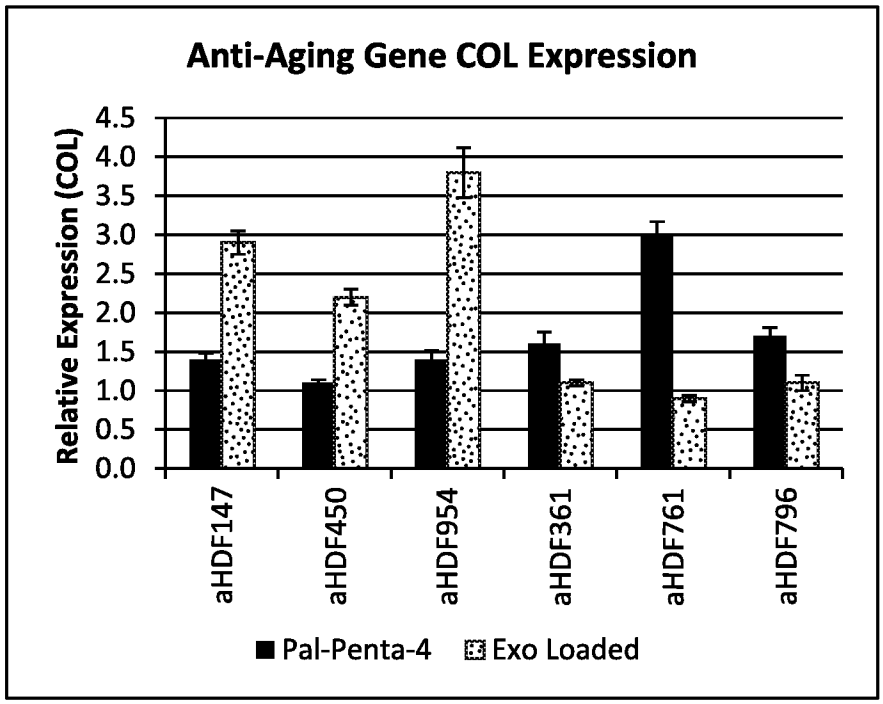
qPCR Analysis (media alone rel exp = 1)

FIG. 4D



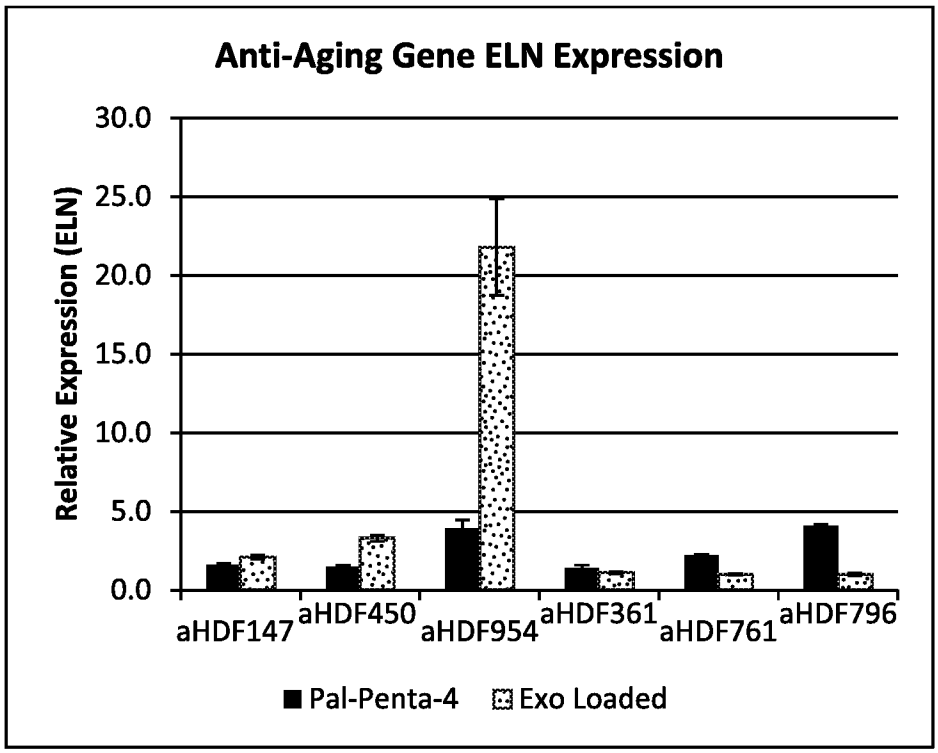
qPCR Analysis (media alone rel exp = 1)

FIG. 4E



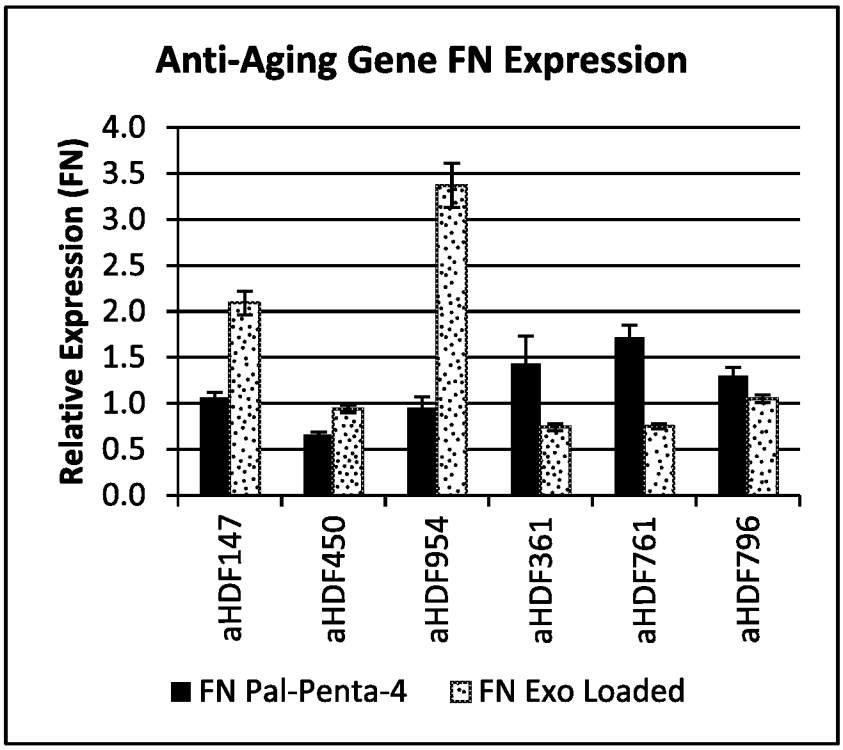
qPCR Analysis (media alone rel exp = 1)

FIG. 5A



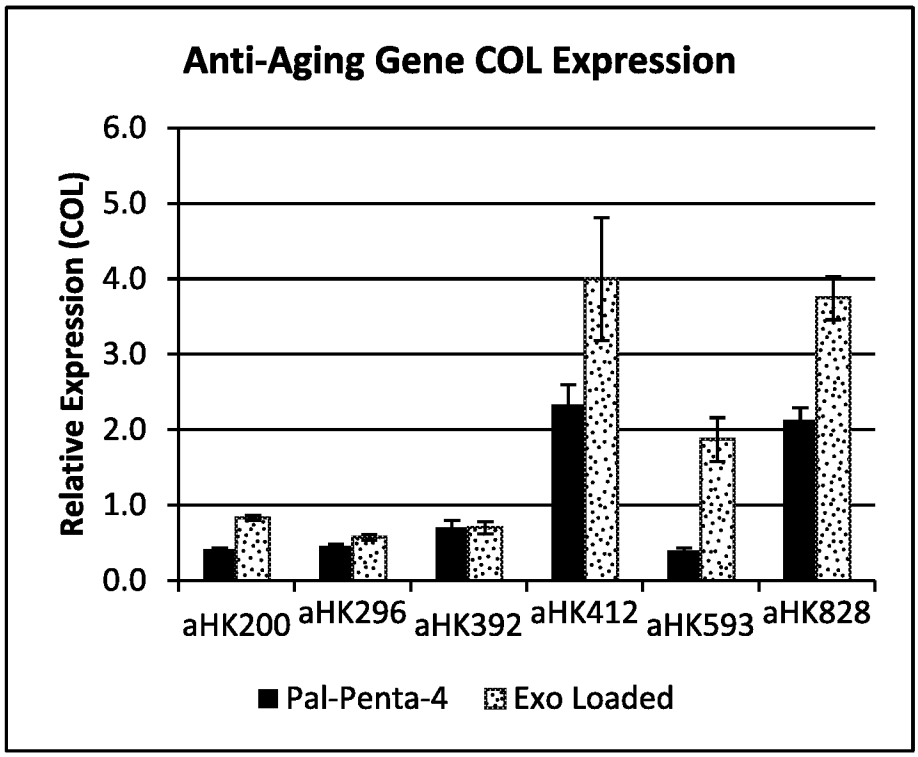
qPCR Analysis (media alone rel exp = 1)

FIG. 5B



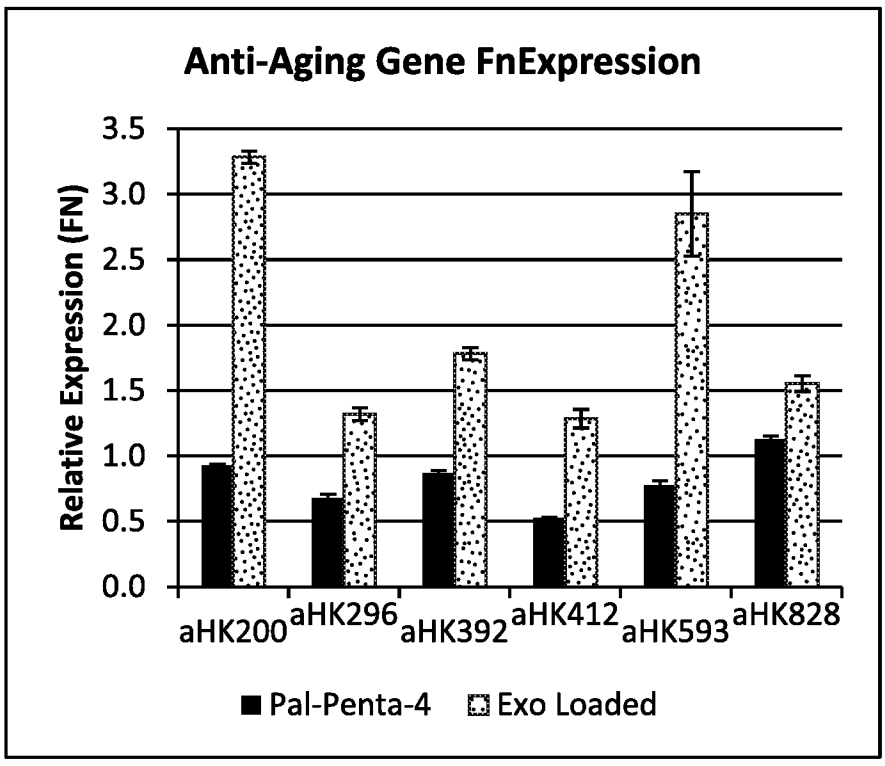
qPCR Analysis (media alone rel exp = 1)

FIG. 5C



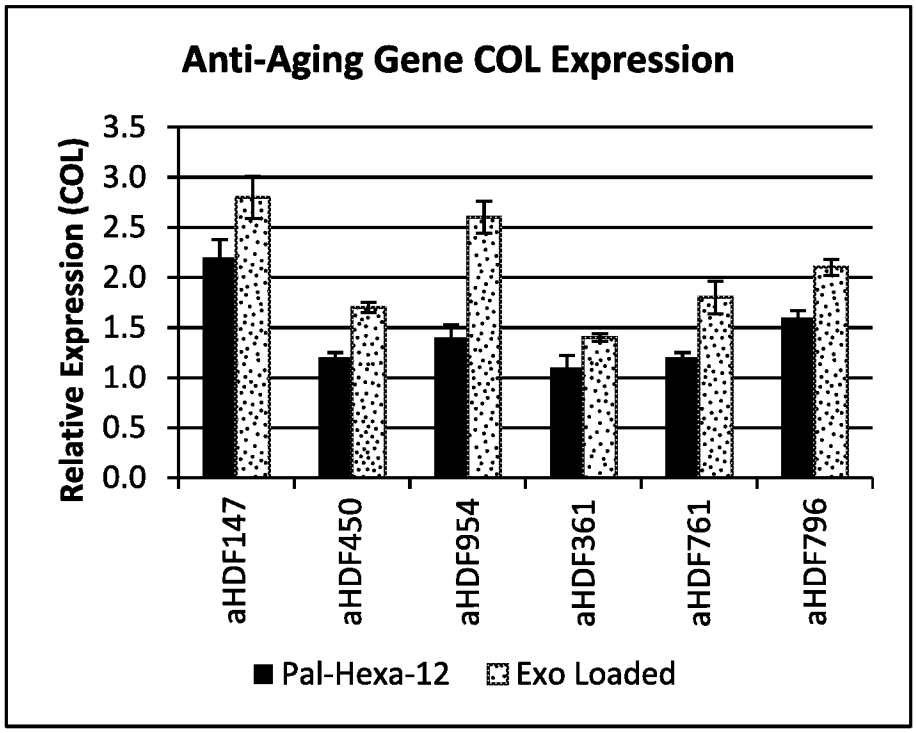
qPCR Analysis (media alone rel exp = 1)

FIG. 5D



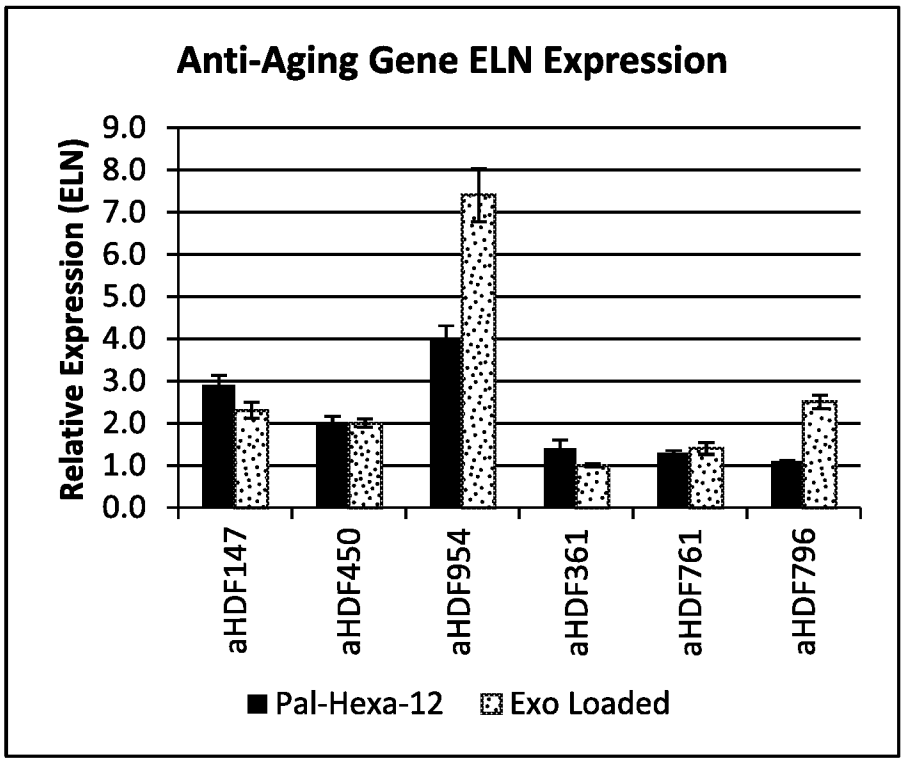
qPCR Analysis (media alone rel exp = 1)

FIG. 5E



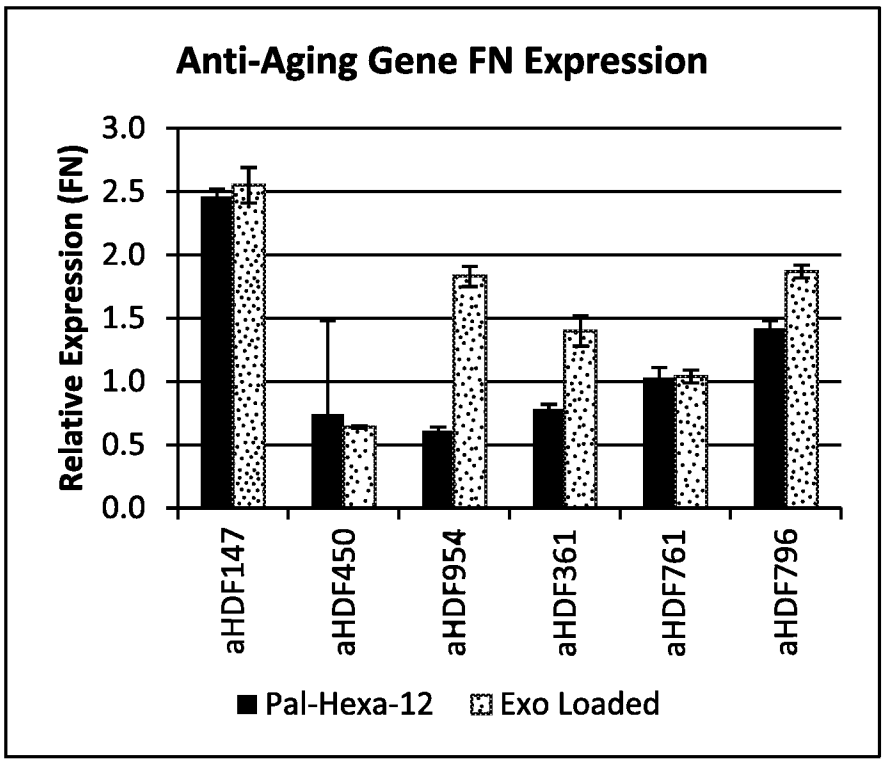
qPCR Analysis (media alone rel exp = 1)

FIG. 6A



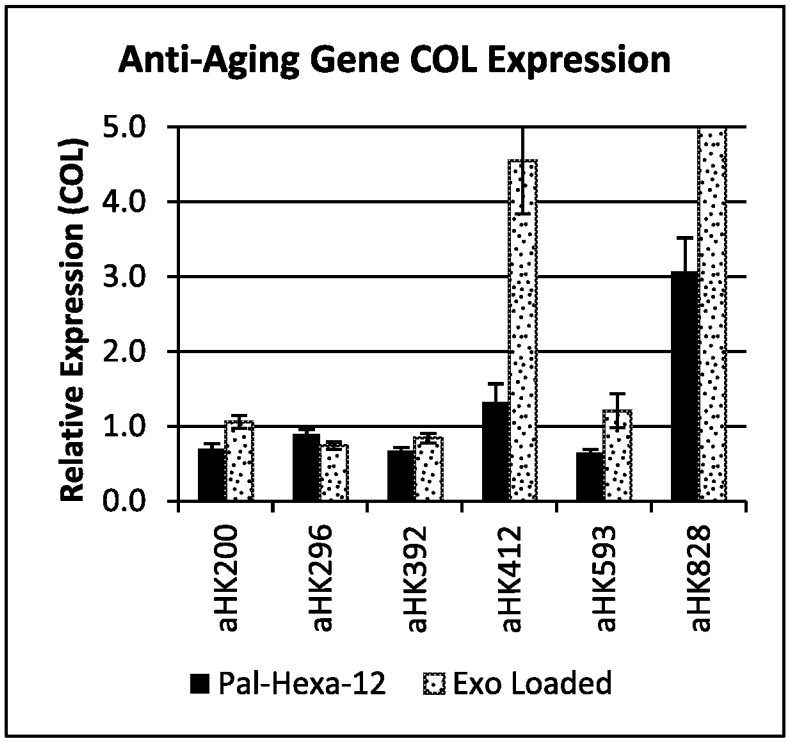
qPCR Analysis (media alone rel exp = 1)

FIG. 6B



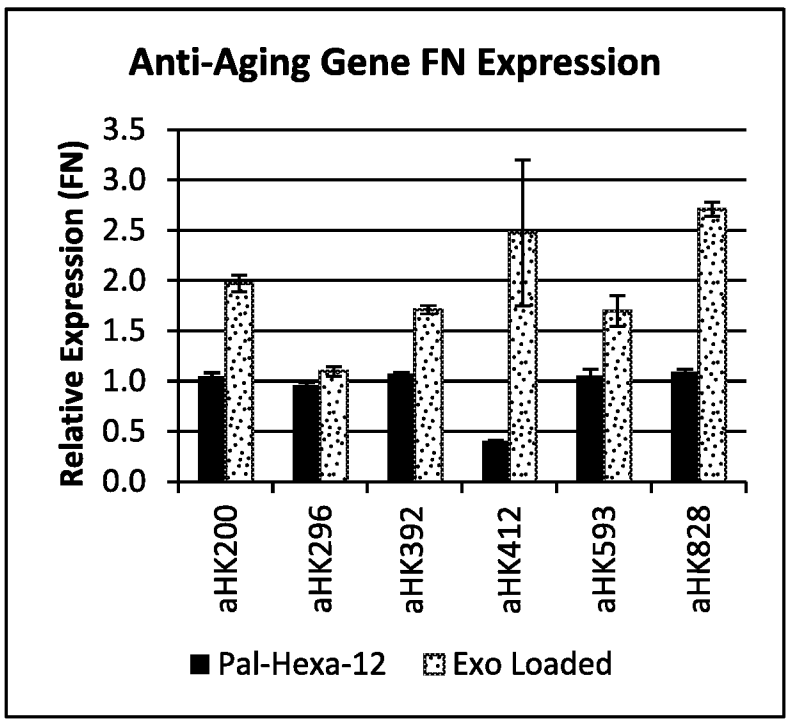
qPCR Analysis (media alone rel exp = 1)

FIG. 6C



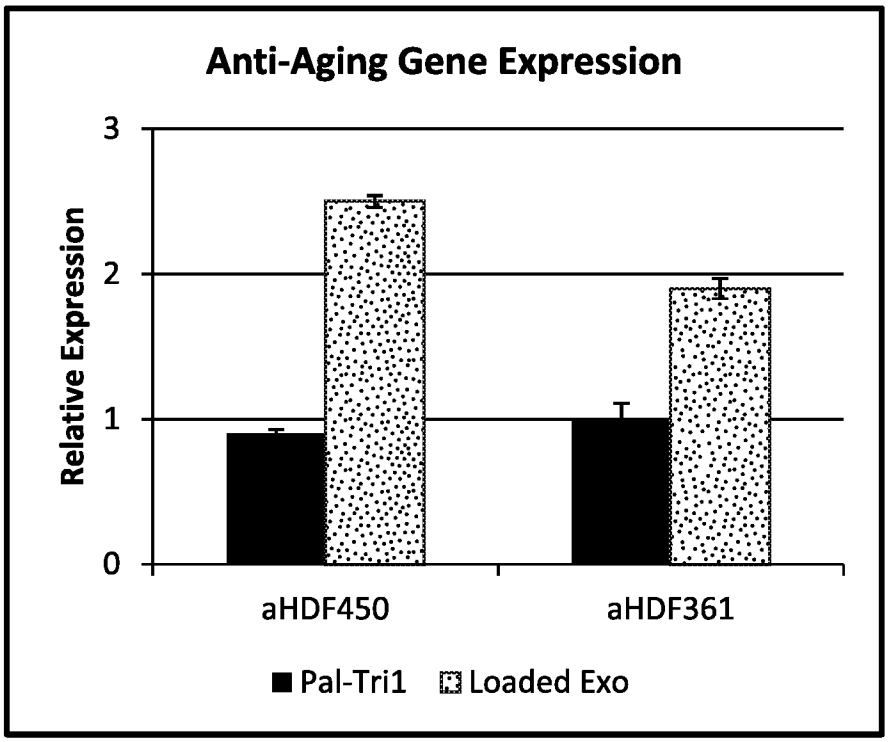
qPCR Analysis (media alone rel exp = 1)

FIG. 6D



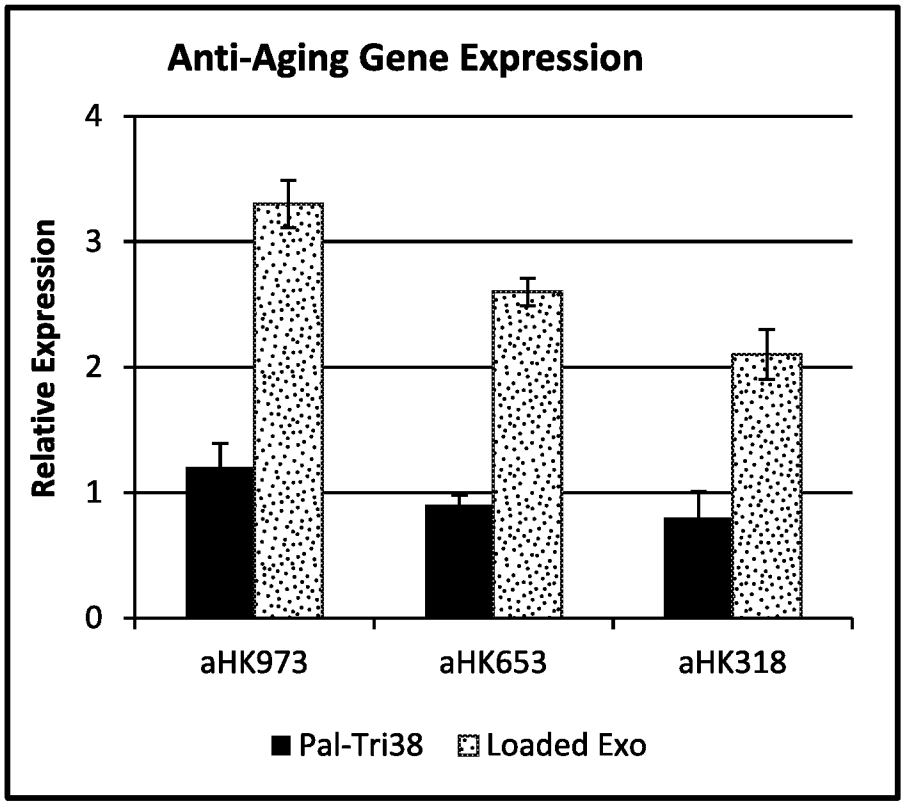
qPCR Analysis (media alone rel exp = 1)

FIG. 6E



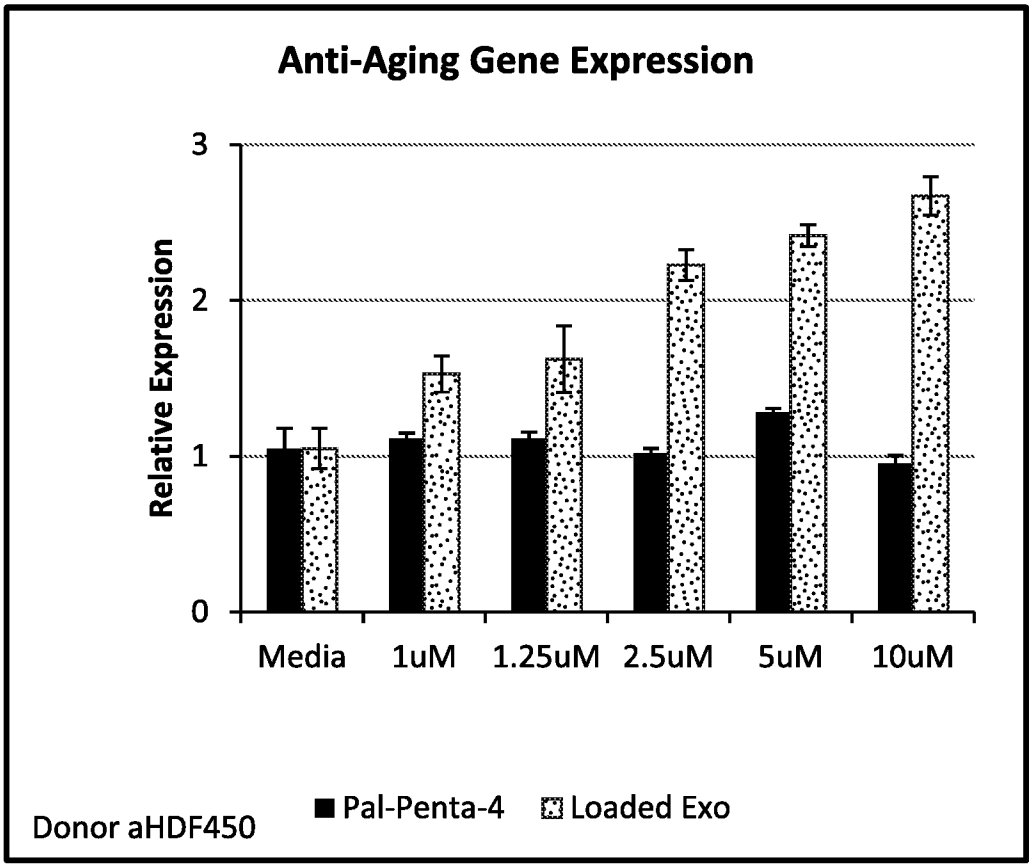
qPCR Analysis (media alone rel exp = 1)

FIG. 7A



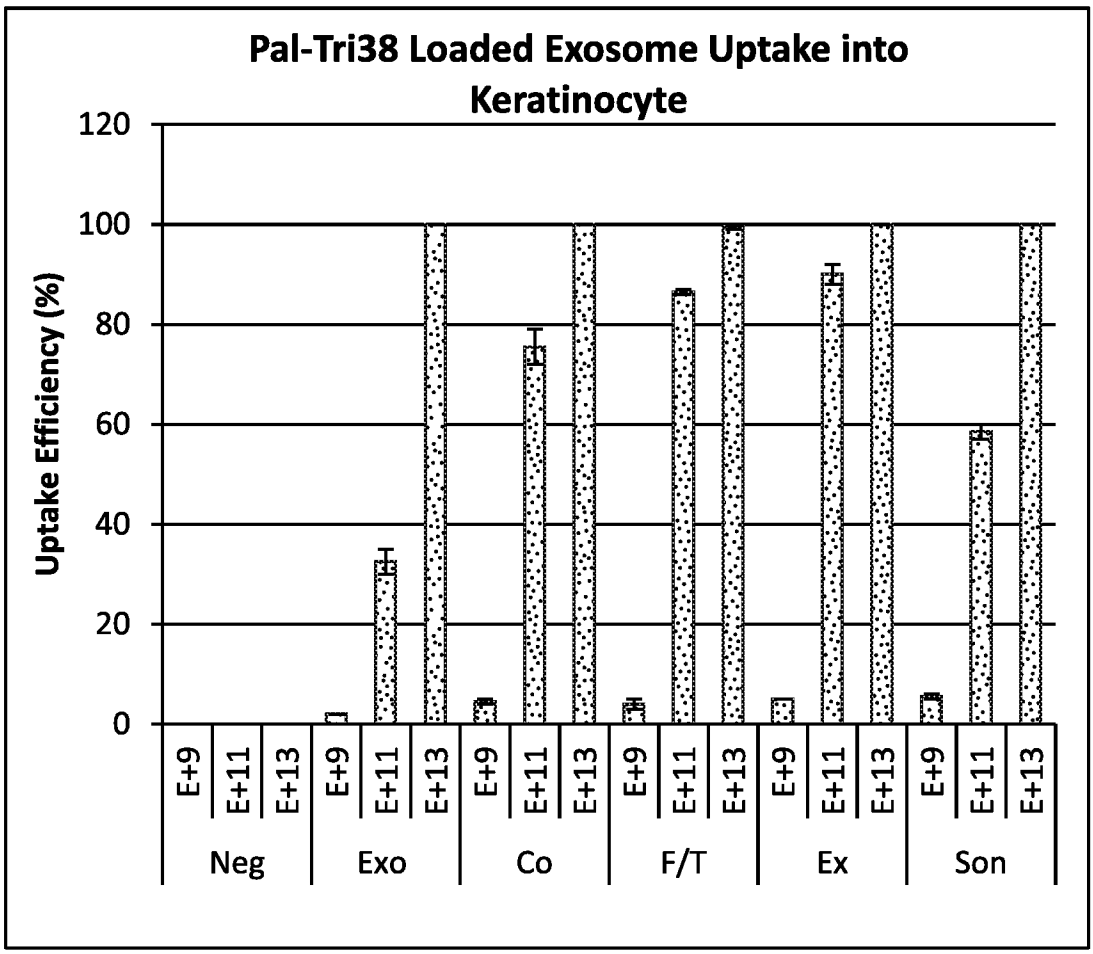
qPCR Analysis (media alone rel exp = 1)

FIG. 7B



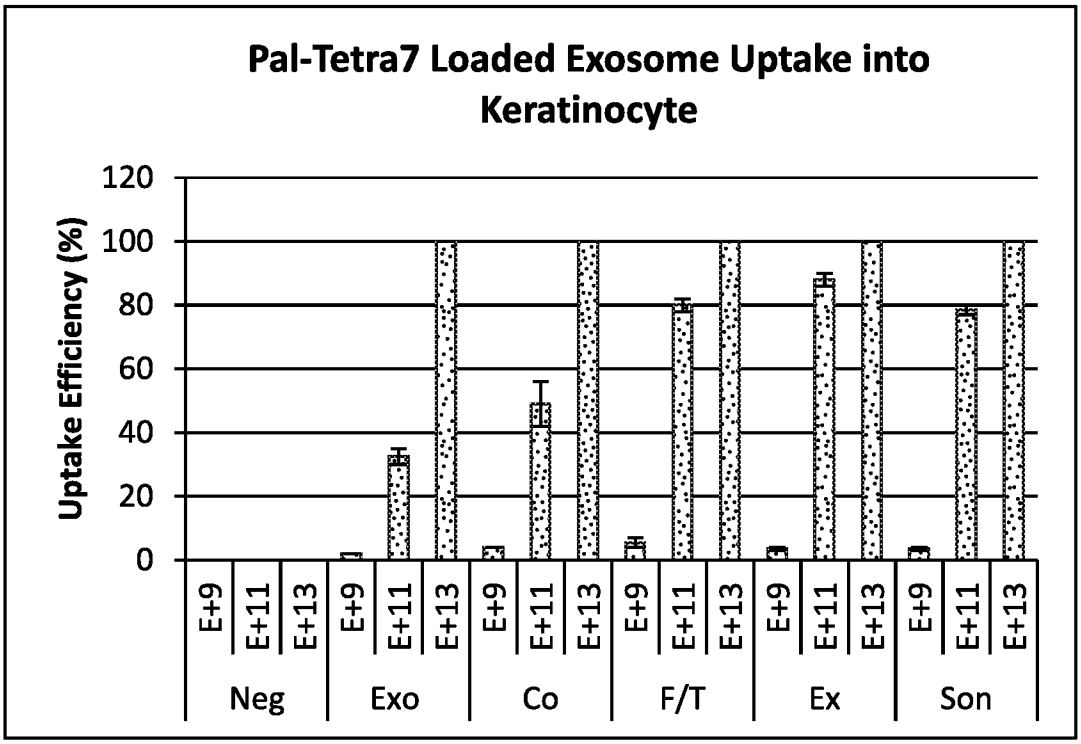
qPCR Analysis (media alone rel exp = 1)

FIG. 7C



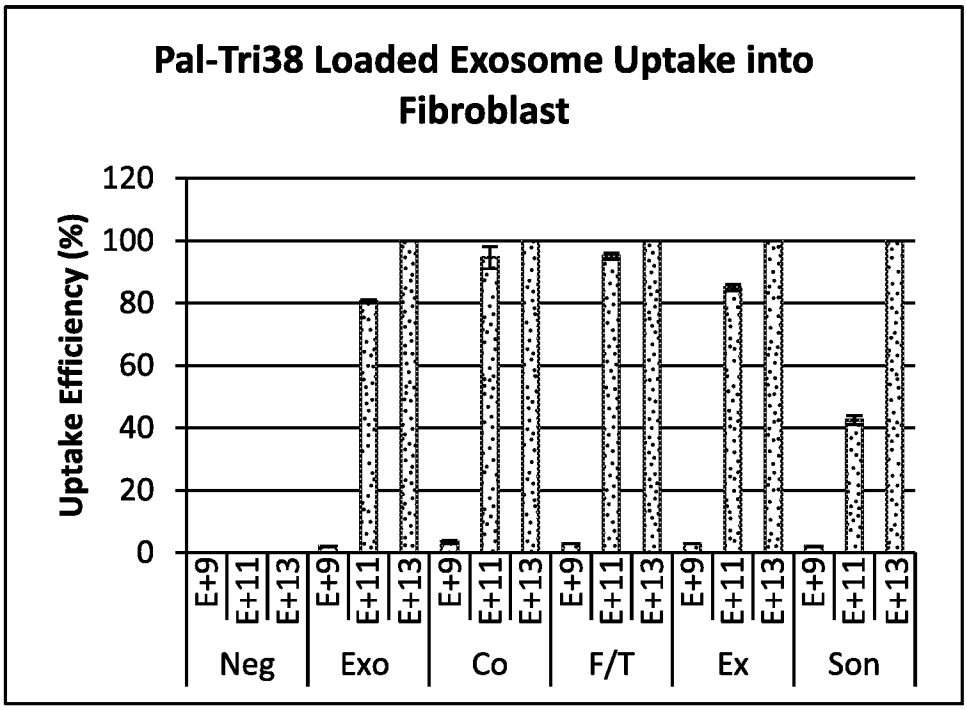
Neg: Negative control (Dio Labeled PBS); Exo: Exosome only labeled with Dio without loading; Co: co-incubation; F/T: Freeze and thaw; Ex: Extrusion; Son: Sonication

FIG. 8A



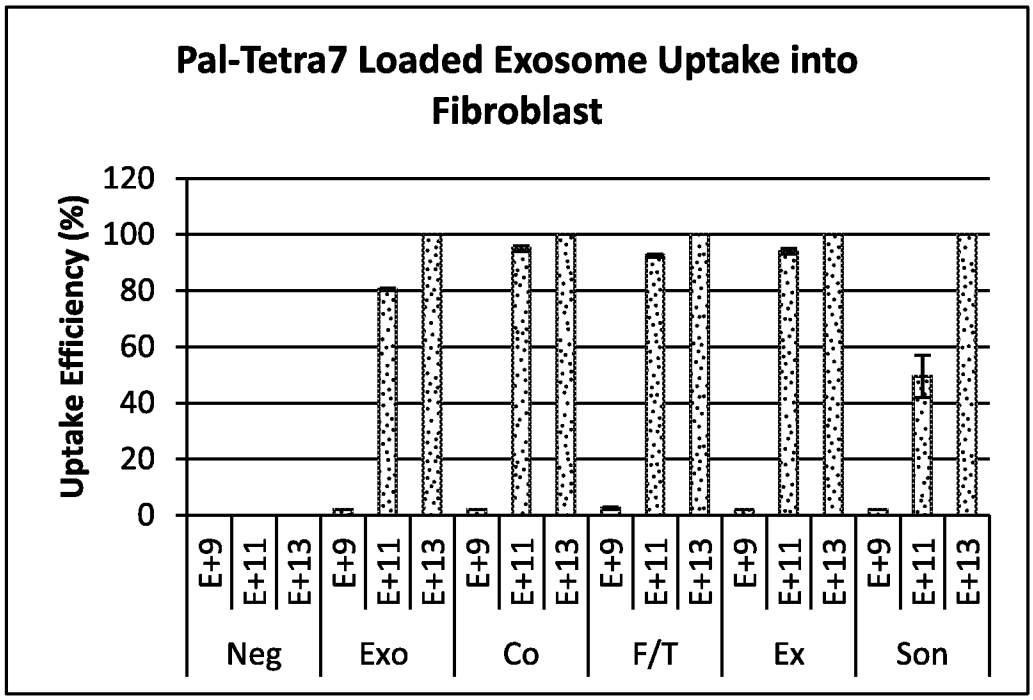
Neg: Negative control (Dio Labeled PBS); Exo: Exosome only labeled with Dio without loading; Co: co-incubation; F/T: Freeze and thaw; Ex: Extrusion; Son: Sonication

FIG. 8B



Neg: Negative control (Dio Labeled PBS); Exo: Exosome only labeled with Dio without loading; Co: co-incubation; F/T: Freeze and thaw; Ex: Extrusion; Son: Sonication

FIG. 8C



Neg: Negative control (Dio Labeled PBS); Exo: Exosome only labeled with Dio without loading; Co: co-incubation; F/T: Freeze and thaw; Ex: Extrusion; Son: Sonication

FIG. 8D

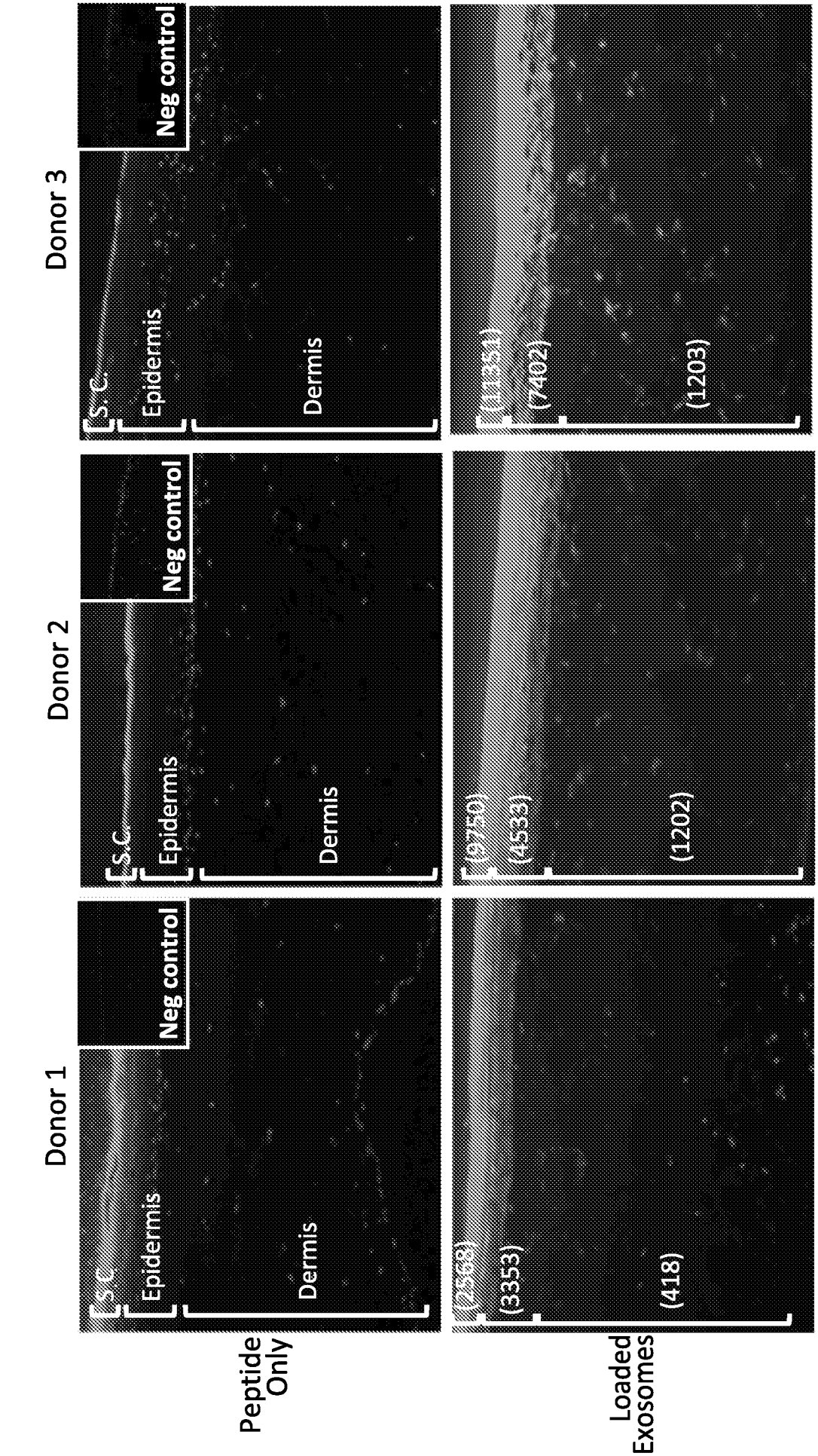
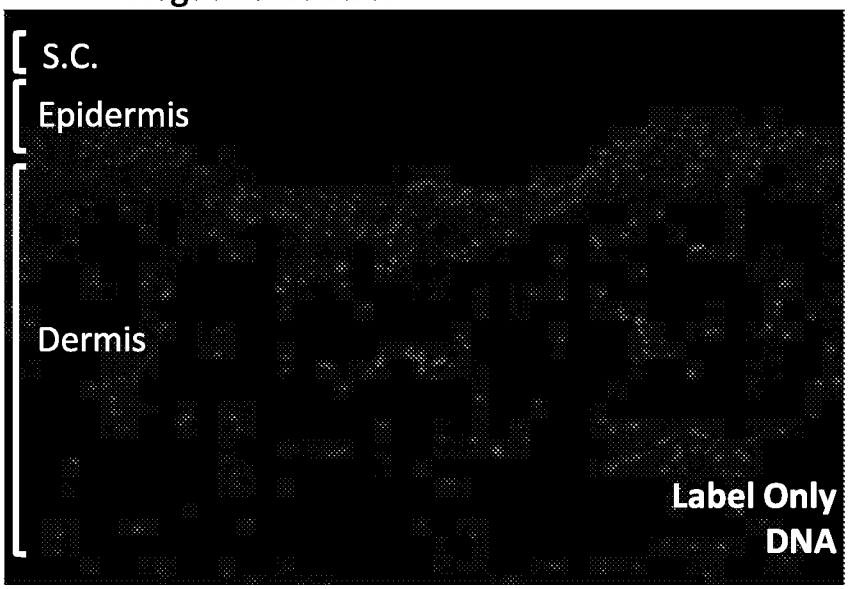
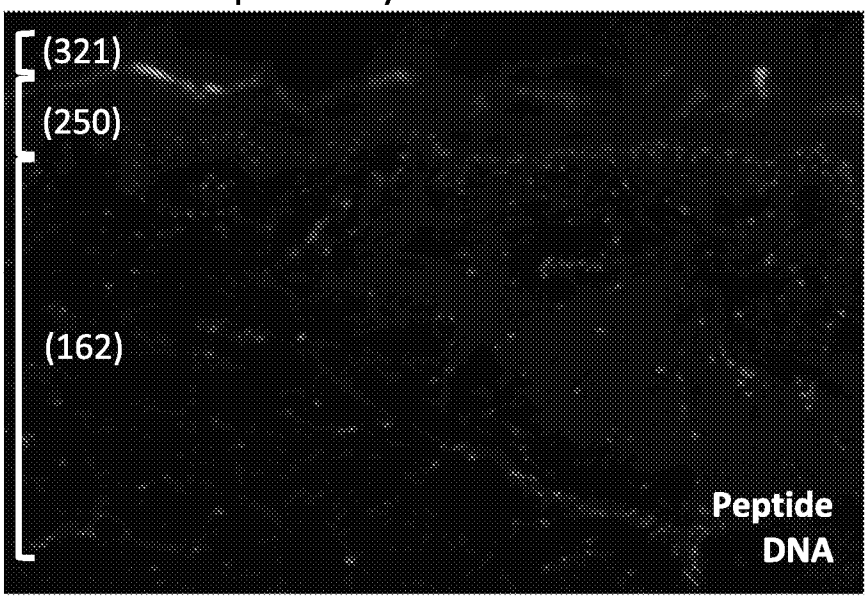

Donor 1
Donor 2
Donor 3
Neg control
S.C.
Epidermis
Dermis
Peptide Only
Loaded Exosomes
(2528)
(3353)
(418)
(9750)
(4533)
(1202)
(11351)
(7402)
(1203)
Microscopic Analysis (Normalized mean intensity in arbitrary units)

FIG. 9A

Negative Control



Peptide Only



Loaded Exosomes

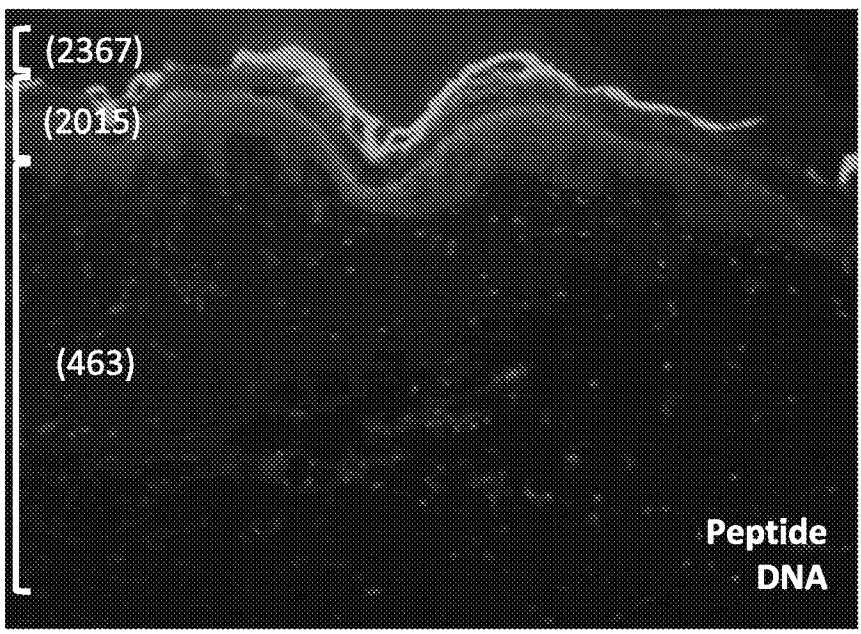


FIG. 9B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/042640

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 8/14; A61K 8/98; A61K 9/48; A61Q 19/08 (2019.01)
CPC - A61K 8/986; A61K 9/0014; A61K 9/5068; A61Q 19/08 (2019.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/450; 514/18.6 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2018/0193270 A1 (PURETECH HEALTH LLC) 12 July 2018 (12.07.2018) entire document	1-3, 20-25 --- 4-9, 26-31
X -- Y	US 2015/0023908 A1 (AL-QAHTANI) 22 January 2015 (22.01.2015) entire document	11-15, 32-40, 42-44, 50-58, 60-62 --- 4, 5, 16-19, 26, 27, 41, 45-49, 59, 63-67
Y	US 2013/0338078 A1 (GALDERISI et al) 19 December 2013 (19.12.2013) entire document	6, 8, 16, 18, 28, 30, 45, 47, 63, 65
Y	US 2014/0301960 A1 (COTY GERMANY GMBH) 09 October 2014 (09.10.2014) entire document	7, 17, 29, 46, 64
Y	US 2017/0231890 A1 (ELC MANAGEMENT LLC) 17 August 2017 (17.08.2017) entire document	9, 19, 31, 48, 66
Y	US 2017/0290758 A1 (BAUMANN) 12 October 2017 (12.10.2017) entire document	41, 59
Y	US 2017/0108503 A1 (CARIS SCIENCE, INC.) 20 April 2017 (20.04.2017) entire document	49, 67

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 December 2019	17 DEC 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/042640

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-5 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/042640

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 10, 68-71
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.